To: Pagan, Ines[Pagan.Ines@epa.gov]
From: Rimer, Kelly
Sent: Fri 10/16/2015 10:09:54 AM
Subject: Fwd: Follow up on chloroprene modeling and additional questions

Ines,

Here is an email from Patrick Walsh. Let's bring in the IRIS folks on this and make it a priority to follow up with Patrick.

Thanks

Kelly Rimer

Leader, Air Toxics Assessment Group

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Begin forwarded message:

From: <PATRICK.A.WALSH@dupont.com>
Date: October 15, 2015 at 6:27:32 PM EDT
To: <Kelly.Petersen@LA.GOV>, <Doris.B.Grego@dupont.com>, <James.B.Allen@dupont.com>, <Carlos.F.Saldana@dupont.com>, <Palma.Ted@epa.gov>, <Morris.Mark@epa.gov>, <Casso.Ruben@epa.gov>, <Rimer.Kelly@epa.gov>, <Strum.Madelcinc@epa.gov>
Subject: RE: Follow up on chloroprene modeling and additional questions

All,

I have reviewed all the appropriate information and my position hasn't changed. I'm worried that EPA is going down the wrong path. Let me explain my thinking to you:

My problem is that the data as presented by EPA with regard to NATA are presented as "cancer risk":

Facility ID	FIPS	Tribal Code	Parameter	Pollutant	Risk Value (cancer risk reported in a million)	Facility Emissions (tpy)	Facility Name	State	County Name	Comme
8026612	22095	Cancer risk	Chloroprene	16	816.044	30.0775	E I DuPont de Nemours & Co - Pontchartrain Site	LA	St. John the Baptist	

(Taken from email from Madeleine Strum to Kelly Petersen, 6/24/15)

That would read to most people that chloroprene is a known, proven human carcinogen. But it hasn't been proven, or even generally accepted, and EPA's own toxicology data states such.

The IRIS database for chloroprene reads similarly to the IARC monograph:

"Under the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), there is evidence that chloroprene is 'likely to be carcinogenic to humans'"

Even the IRIS group will not explicitly state that chloroprene is a KNOWN human carcinogen. The entire series of documents discusses chloroprene's carcinogenicity in mice and rats **only**. While they can be used as models for human physiology, mice and rats are NOT human, and there are numerous examples of materials that are spectacularly toxic to non-human animals but have little or no effect on humans (chocolate springs to mind). Therefore, it is, in my opinion, an irresponsibly large leap to present the chloroprene release data as definitely carcinogenic to humans by presenting it as "increased cancer risk".

In addition, the epidemiological data does not comport with the model at all. The following table describes actual cancer rates for St. John Parish for the most recent 4-year period for which data is available:

Rank	County	Annual Incidence Rate(†) over rate period - cases per 100,000	Lower 95% Confidence Interval	Upper 95% Confidence Interval	Average Annual Count over rate period	Rate Period	Recent Trend	Recent 5-Year Trend (‡) in Incidence Rates	Lower 95% Confidence Interval	Upper 95% Confidence Interval
53	St. John the Baptist Parish(7,9)	460.8	432.3	490.7	209	2008-2012	stable	-2.2	-9.4	5.6

(Data from

http://statecancerprofiles.cancer.gov/incidencerates/index.php?stateFIPS=22&cancer=001&race=00&sex=0&age=001

Given the following:

1. 50+ year history making chloroprene in St. John Parish
2. 20-30 year latency period for most cancers

According to the risk factors EPA attributes to our chloroprene emissions, St. John Parish should have the highest cancer rate in the state. This should be especially true given that our history of emitting chloroprene is much longer than the typical latency for cancer. But in actuality, St. John is in the **lowest quartile** of measured cancer rates in the state (#53 out of 66 parishes) and the rate of cancer is decreasing according to the 5-year trend. Thus, the model has a serious flaw as it doesn't come close to reflecting real, published cancer rate data.

The above, taken together, indicate that EPA is planning to publish misleading data in an inflammatory way. Therefore, it would be irresponsible to publish it. I strongly urge EPA to reconsider its present course.

Patrick A. Walsh, CIH
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Let's Solve. DUPONT

-----Original Appointment-----
From: Kelly Petersen [mailto:Kelly.Petersen@LA.GOV]
Sent: Tuesday, October 06, 2015 10:09 AM
To: Kelly Petersen; GREGO, DORIS B; ALLEN, JAMES B; SALDANA, CARLOS F; Palma, Ted; Morris, Mark; Casso, Ruben; 'Rimer, Kelly'; Strum, Madeleine; WALSH, PATRICK A.
Subject: Follow up on chloroprene modeling and additional questions
When: Tuesday, October 06, 2015 11:00 AM-12:00 PM (UTC-06:00) Central Time (US & Canada).
Where: `DEQ/Room 919 - OMF Conference

Please join a conference call at 11am central time on Tuesday, October 6th. The call in information is below.

Meeting Number: Ex. 6 - Personal Privacy
To join the conference call:
Ex. 6 - Personal Privacy
(2) Enter the Meeting Number, then #
Thanks, Kelly Petersen

To: Casso, Ruben[Casso.Ruben@epa.gov]
Cc: 'Ted Palma'[Palma.Ted@epa.gov]; Strum, Madeleine[Strum.Madeleine@epa.gov]
From: Rimer, Kelly
Sent: Thur 10/1/2015 7:45:08 PM
Subject: NATA Chloroprene to Ruben 10 01 15.pptx
NATA Chloroprene to Ruben 10 01 15.pptx

Hi Ruben,

As promised here are a few slides on the chloroprene issue, for you to use to brief your upper management. I'll see about LA setting up a meeting on the issue, and we will go from there.

Thanks,
Kelly

Regulatory Toxicology and Pharmacology 70 (2014) 203–213

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

A constrained maximum likelihood approach to evaluate the impact of dose metric on cancer risk assessment: Application to b-chloroprene

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article info

Article history:
Received 21 March 2014
Available online 7 July 2014

Keywords:
Physiologically based kinetic modeling
Constrained likelihood approach
b-Chloroprene

abstract

b-Chloroprene (2-chloro-1,3-butadiene, CD) is used in the manufacture of polychloroprene rubber. Chronic inhalation studies have demonstrated that CD is carcinogenic in B6C3F1 mice and Fischer 344 rats. However, epidemiological studies do not provide compelling evidence for an increased risk of mortality from total cancers of the lung. Differences between the responses observed in animals and humans may be related to differences in toxicokinetics, the metabolism and detoxification of potentially active metabolites, as well as species differences in sensitivity. The purpose of this study was to develop and apply a novel method that combines the results from available physiologically based kinetic (PBK) models for chloroprene with a statistical maximum likelihood approach to test commonality of low-dose risk across species. This method allows for the combined evaluation of human and animal cancer study results to evaluate the difference between predicted risks using both external and internal dose metrics. The method applied to mouse and human CD data supports the hypothesis that a PBK-based metric reconciles the differences in mouse and human low-dose risk estimates and further suggests that, after PBK metric exposure adjustment, humans are equally or less sensitive than mice to low levels of CD exposure.

1. Introduction

b-Chloroprene (CD, CAS# 126-99-8, 2-chloro-1,3-butadiene) is a compound used in the manufacture of polychloroprene rubber. Chronic inhalation studies in animals have demonstrated that CD is carcinogenic in B6C3F1 mice and Fischer 344 rats in multiple target organs (lung, liver, circulatory systems, forestomach, Harderian gland, kidney, mammary gland, mesentery, oral cavity, skin, and thyroid gland) (Melnick et al., 1999; National Toxicology Program, 1998). In addition, respiratory and liver cancers have been associated with CD exposure in several epidemiological studies (Acquavella and Leonard, 2001); however, interpretation of these findings has been difficult due to methodological limitations, including the inability to assign quantitative values for CD exposures, the small number of observed outcomes, and the small sample sizes for occupational studies (Marsh et al., 2007a). This makes the comparison of estimates of risk based on animal versus human results difficult.

While epidemiological studies are available for chloroprene, due to the uncertainties in the epidemiological studies the most recent quantitative risk assessment conducted by the USEPA (2010) used only animal data. The resulting cancer unit risk is driven by the most sensitive endpoint in animals, the incidence of lung tumors in female mice. Integration of the epidemiological studies does not provide compelling evidence for an increased risk of mortality from total cancers of the lung following inhalation exposure to chloroprene (Marsh et al., 2007a,b).

Previous studies have examined differences in toxicokinetics between animals and humans to determine if this is potentially

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http://dx.doi.org/10.1016/j.yrtph.2014.07.001
0273-2300/

the contributing factor to the differences in response between animals and humans. The initial step in metabolism is oxidation forming a stable epoxide, (1-chloroethenyl) oxirane, a genotoxicant that might be involved in the observed carcinogenicity in animals (Himmelstein et al., 2004b). Differences between the responses observed in animals and humans may be related to differences in toxicokinetics, to the metabolism and detoxification of potentially active metabolites (Himmelstein et al., 2004a,b), as well as to differences in species sensitivity. Specifically, Himmelstein et al. (2004a) found that the oxidation (Vmax/Km) of CD in liver was slightly faster in rats and mice than in humans and hamsters, and in lung microsomes was much greater for mice compared to other species. In addition, hydrolysis (Vmax/Km) of (1-chloroethenyl) oxirane, in liver and lung microsomes, was faster for humans and hamsters than for rats and mice.

In current risk assessments for chloroprene (USEPA, 2010), external exposure estimates are relied upon, which does not consider species differences in toxicokinetics. These differences may be critical in characterizing the potential risk of cancer following exposure to chloroprene, especially if the generation of a metabolite is related to the potential for cancer risk. The availability of physiologically based kinetic (PBK) models for both mice and humans (Yang et al., 2012) provides a unique opportunity for comparison of animal and human risk estimates based on external and internal exposure metrics. The PBK model for chloroprene incorporates the available data regarding species differences in metabolism of chloroprene. Application of the model allows for species-specific estimation of internal exposure metric, specifically the amount of chloroprene metabolized per gram of lung tissue. Risk estimates can then be compared across species based on this equivalent internal exposure metrics rather than external air concentrations.

The purpose of this study was to develop and apply a novel method that combines the results from available PBK models for chloroprene with a statistical maximum likelihood approach to test commonality of low-dose risk across species. This method allows for the combination of human and animal cancer study results to evaluate the difference between risk estimates obtained using both external and internal dose metrics.

The maximum likelihood approach applied allows for the evaluation of the ability of traditional dose–response models, such as the Multistage model, to describe the response pattern under the constraint of equal risk at a dose of interest (either internal or external), specifically a possible point of departure (POD). The results provide a demonstration of which dose metric provides statistically equivalent human- and animal-based risk estimates. Additional analyses were also conducted to investigate the impact of uncertainty in the estimated exposure levels for the human occupational study and to address the question of potential cross-species pharmacodynamic differences.

2. Material and methods

The method described here requires both animal data (a well-conducted two-year bioassay) and epidemiological data sufficient to allow dose-response analysis. Rather than modeling them separately, the approach adopted is to jointly model the selected studies to determine if, and under what circumstances, risk estimates of interest can be determined to be consistent across species. Jointly modeling the data requires software that allows for constrained maximization of the combined likelihood of the animal and human dose–response relationships with testing of hypotheses based on the comparison of the constrained maximum likelihood to the unconstrained (separate) likelihoods for the two species. Fig. 1 depicts the overall procedure.

2.1. Animal data

A two-year inhalation study of CD was conducted in F344/N rats and B6C3F$_1$ mice (National Toxicology Program, 1998). This is the bioassay relied upon by the Environmental Protection Agency (EPA) in the recent CD Integrated Risk Information System (IRIS) assessment (USEPA, 2010). Groups of 50 males and 50 females were exposed by inhalation for 6 h per day 5 days per week for 2 years to 0, 12.8, 32 or 80 ppm of CD. The National Toxicology Program (NTP) (1998) concluded that there was clear evidence of carcinogenicity in both the rats and mice following inhalation exposure to CD. In the F344/N rats, this conclusion was based on the increased incidences of neoplasms of the thyroid gland and kidney in males and females, increased incidences of neoplasms in the lung in males only and in the oral cavity and mammary gland in females only. In the B6C3F$_1$ mice, the conclusion of clear evidence of carcinogenicity was based on the increased incidence of neoplasms in the lung, circulatory system, forestomach and Harderian gland in both sexes, in the kidney for males only and the mammary gland, liver and skin for females only (see Table 5-4 in USEPA, 2010).

Based on the NTP (1998) results, USEPA (2010) concluded that that mouse is the most sensitive species, due to the increased tumor incidence and multisite distribution in the mouse relative to the rat. The EPA calculated a composite unit risk from all the female mice cancer endpoints listed above (9.8×10^{-1} per ppm; 2.7×10^{-4} per $\mu g/m^3$), and the unit risk estimated from the combined incidence of lung adenomas or carcinomas in the female mice produced the highest site-specific unit risk (6.4×10^{-1} per ppm; 1.8×10^{-4} per $\mu g/m^3$). As it was the most sensitive of the site-specific endpoints, combined lung adenomas and carcinomas is the endpoint considered in the current analysis. Analyses of rat responses, and perhaps additional mouse responses, may follow, given the success of this investigation.

2.2. Human data

Marsh et al. (2007a,b) conducted a historical cohort study to investigate the mortality of industrial workers potentially exposed to CD and other substances (including a potential confounding co-exposure to vinyl chloride). This study represents one of the most recent epidemiological studies and the design attempted to address the problems identified with earlier studies by conducting a detailed exposure assessment for both chloroprene and vinyl chloride monomer. The emphasis of the study was on cancer mortality, including respiratory system cancer. Four different CD production sites (i.e., Louisville, KY; Pontchartrain, LA; Maydown, Northern Ireland; and Grenoble, France) were included in the Marsh et al. study. The Louisville cohort examined by Marsh et al. (2007a,b) had the greatest number of exposed individuals, the greatest number of person-years of follow-up, and the greatest average exposure level (both in terms of the intensity level, ppm, and in terms of cumulative exposure, ppm-years). The greater exposure levels, combined with the greatest number of exposed individuals, increase the probability of detecting any carcinogenic effect following exposure to CD. Respiratory system cancer mortality from the Louisville cohort was used in this analysis as those data came from the best epidemiological dataset available (in terms of adequacy of size and suitability for dose–response analysis) that measured an endpoint that was comparable to the most sensitive endpoint in mice. The other cohorts may be subject to future analyses; inclusion of additional cohorts may increase the power of the epidemiological modeling.

For the Louisville cohort, approximate quartiles of the data were determined by Marsh et al. (2007b) based on the distribution of death from all cancers, and these quartiles were used to define

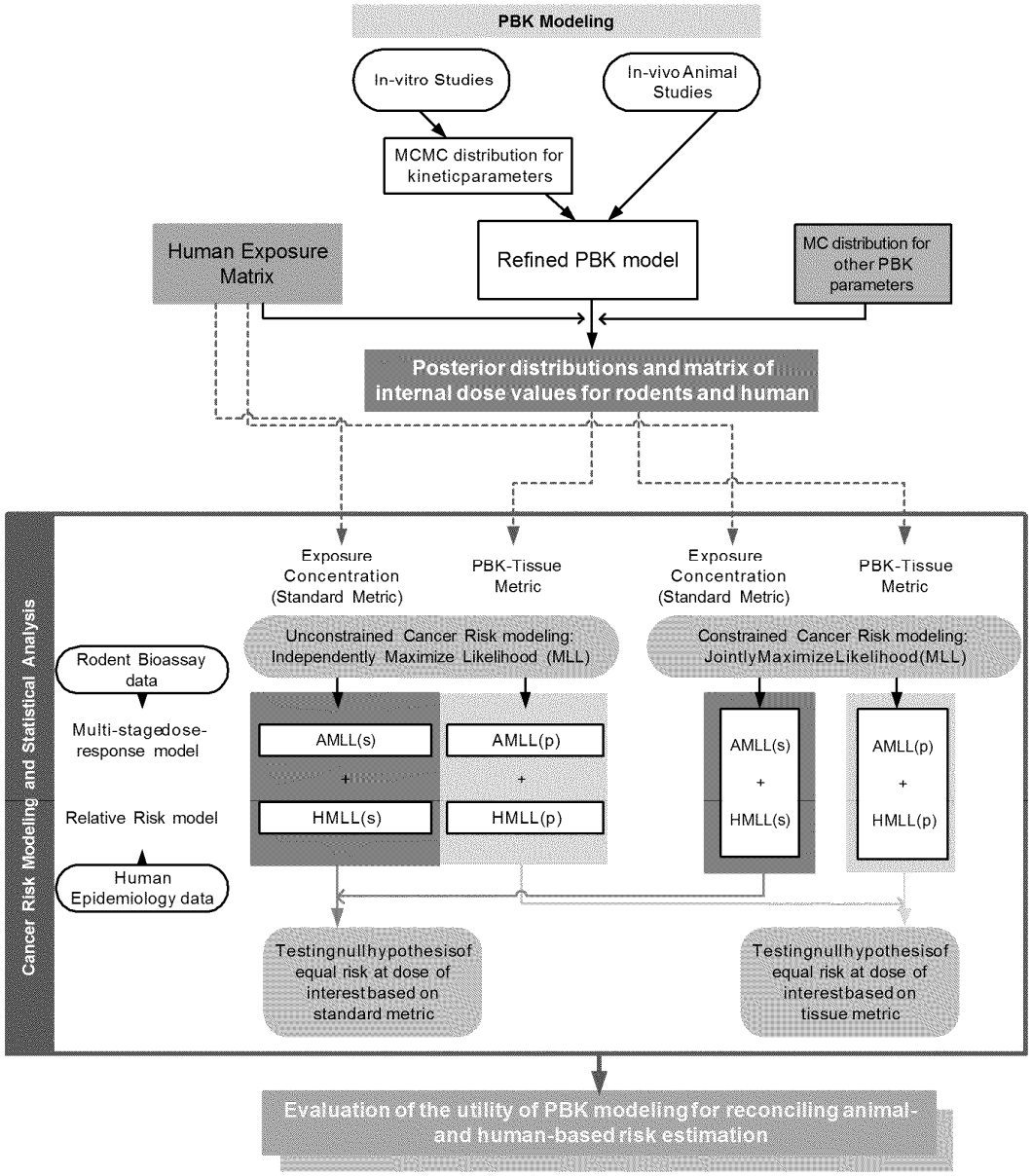

Fig. 1. Overview of physiological based kinetic modeling probabilistic dose response modeling.

the subgroups for all other cancer types, including the respiratory cancer used in this analysis. The exposure reconstruction detailed in Esmen et al. (2007b) was used, in combination with the Occupational Cohort Mortality Analysis Program (OCMAP) (described in detail in Marsh et al., 1998) to determine the quartile-specific and overall average cumulative exposure.

2.3. Estimation of exposure/dose

In the evaluation of the animal data, external air concentrations used in the exposure-response modeling were the administered air concentrations in the NTP (1998) study in ppm adjusted to an equivalent continuous exposure, adjusting for hours per day (6/24) and days per week (5/7) (Table 1). Similarly, the human cumulative doses were adjusted from occupational to continuous exposure by adjusting for the number of work weeks per year (50/52), for work days per week (5/7) and for percentage of total daily inhalation that occurs during work hours (10/20) (USEPA, 2009). Adjusted values are shown in Table 2.

Based on the range of reported exposures for each quartile, the midpoints of cumulative exposure for the first three exposure groups were used (assumed to characterize the respective group average exposure for dose–response modeling). However, because the high exposure group was characterized as 164.053+ ppm-years with no highest exposure value, an approach was needed to characterize the average exposure for this group (Table 2). The average exposure used for the highest group was calculated based on the midpoint values for exposure groups 1 through 3, the overall average cumulative exposure computed by OCMAP, and the number of person-years apportioned to each group, shown here:

Table 1
Animal data modeled via the multistage model.

Dose group	Continuous exposure equivalent (ppm)	PBK metric (lmole/g-lung/day)	Group size	Number of animals with respiratory system cancer
1	0	0	50	4
2	2.3	0.705	49	28
3	5.7	1.12	50	34
4	14.3	1.47	50	42

Table 2
Human data modeled via a linear relative risk model.

Cumulative exposure group	Published cumulative exposure ranges (ppm-years)	Average cumulative exposure (ppm-years)	Assumed adjusted average cumulative exposure (ppm-years)	PBK metric (lmole of metabolite/g lung/day-years)	Person years of observation	Deaths from respiratory system cancer	SMR	Computed expected
1	<4.747	2.37	0.814	0.0083	68918	62	0.71	87.32
2	4.747–55.918	30.3	10.4	0.107	56737	67	0.71	94.37
3	55.918–164.052	110	37.8	0.387	39840	77	0.92	83.70
4	164.053+	297[a]	102	1.05	32424	60	0.65	92.31

[a] Calculated using text Eq. (1).

$$\text{ppm-years}(\text{avg},\text{total}) = \sum_{i} \text{ppm-years}(\text{avg},i) \cdot \text{PY}(i) / \text{PY}(\text{total}) \quad (1)$$

where ppm-years(avg, total) is the average cumulative exposure for the entire cohort (80.35 ppm-years), ppm-years(avg, i) is the assumed average cumulative exposure for groups 1-3 or the unknown X ppm-years for group 4; PY(total) is the total number of person years of follow-up for the cohort (197919); and PY(i) is the person years of follow-up for group i (68918, 56737, 39840, and 32424 years for groups 1 through 4, respectively). The values for the ppm-year ranges and person years of follow-up (see also Table 2) are from Marsh et al. (2007b). The only unknown in the equation above, X, is for the ppm-years for group 4. Solving for X gives an estimate of the cumulative exposure for group 4 of 297 ppm-years.[1]

An internal dose metric (PBK metric) was estimated for both the animal and human datasets using the PBK model by Yang et al. (2012). Following Markov Chain Monte Carlo (MCMC) analyses, Yang et al. derived a set of posterior distributions for each of the kinetic parameters in both the mouse and the human PBK models. The mean from each distribution (i.e., one for each kinetic parameter) as well as the standard physiological and partition coefficient values (Yang et al., 2012) for each species were used in the corresponding PBK model to derive the internal dose metric of lmoles of metabolized CD/g lung/day for each exposure group in both the mouse experimental study and the human occupational study. Such a metric reflects the estimated metabolism of CD to reactive metabolites, including (1-chloroethenyl) oxirane, which are the proposed carcinogenic moieties (Yang et al., 2012). Since metabolism of CD is different between mice and humans, the use of PBK model estimates of internal dose, as a measure of exposure, provides a method to account for these species-specific differences.

For both the mouse and the human, the models were run for a week-long exposure (5 days per week). It was observed that after the 2 (weekend) days of non-exposure, chloroprene was cleared from the body for both species. Thus, a single week of modeling the experimental exposures or occupational exposures was sufficient to calculate the lifetime daily average.

2.4. Calculation of animal-based risks

For the current assessment, the Multistage model provided in the USEPA Benchmark Dose Software (BMDS) program (USEPA, 2012) was fit to the female mice lung adenoma or carcinoma incidence data using the continuous exposure equivalent in ppm (adjusted from 6 h per day 5 days per week to continuous). In addition, the model was also fit to the data using the internal PBK metric of lmole CD metabolized/g of lung/day obtained from simulations of the Yang et al. (2012) PBK model (Table 1).

The multistage model has the mathematical form:

$$P(d) = 1 - e^{-(q_0 + q_1 d + \ldots + q_k d^k)} \quad (2)$$

where d is the average lifetime daily dose, P(d) is the lifetime probability of tumor from the dose level d, and $q_0, \ldots, q_k$ are nonnegative parameters estimated by fitting the model to experimental animal data. The multistage modeling performed in this analysis assumed k = 2, i.e., it used a two-stage model.

The multistage model is a flexible statistical model that can describe both linear and non-linear dose-response patterns. It has been used as the standard for cancer risk analysis, and for many years the default dose-response model for federal and state regulatory agencies in the United States for calculating quantitative estimates of low-dose carcinogenic risks from animal data (USEPA, 1986, 2005).

The choice of a low-dose extrapolation method used by the EPA, in particular, in dose–response assessments should be informed by the available information on the mode of action of cancer, as well as other relevant biological information, and not solely on goodness-of-fit to the observed tumor data (USEPA, 1992). However, when data are limited or when uncertainty exists regarding the mode of action, models which incorporate low-dose linearity are the default approach. EPA usually employs the linearized multistage procedure in the absence of adequate information to the contrary; many of the available IRIS values are based on the results from this model. In that capacity, it is regularly used on data sets with only a few data points as is common for animal studies.

Using the external and internal dose metrics for CD, a single maximized log-likelihood was determined for each: the

[1] This approach used to determine the average concentration for the highest exposure group was deemed preferable to using a midpoint between 164 ppm-years and 1351.5 ppm-years, the reported maximum seen in the cohort. The dose for the highest group would have been larger (758 ppm-years) and would not have maintained the reported average ppm-year value for the entire cohort. Rather than relying upon a midpoint of the range of exposure, the consideration of average values for grouped exposure summaries in the current approach reflects all of the available information regarding cohort exposure.

unconstrained animal maximum log-likelihood for the standard (or external) metric (AMLLs) and the unconstrained maximized log-likelihood for the internal metric (AMLLp) (Fig. 1). Each of the AMLLx values represents the usual data-specific measure of the fit of the model to the animal bioassay results and is the maximum value of that log-likelihood with no other constraints.

2.5. Calculation of epidemiology-based risks

A linear relative risk model was fit to the summarized data from the Louisville cohort used in this analysis (Table 2).[2] The assumed average cumulative exposure, the observed deaths from respiratory system cancer, and the expected deaths from respiratory cancer were used in a linear model to estimate the relative risk:

$$\text{Relative Risk} = \text{Observed}/\text{Expected} = a \cdot (1 + bd) \quad (3)$$

where d is a measure of cumulative exposure and a and b are parameters to be estimated. "Expected" was computed as the observed number of cases ("Observed") divided by the Standardized Mortality Ratio (SMR). Fitting to the human epidemiological data (Table 2) was accomplished via Poisson maximum likelihood techniques (Frome, 1983). The log-likelihood for the assumed Poisson distribution in a group having cumulative exposure d is expressed as:

$$LL = -\text{Expected} \cdot a \cdot (1 + bd) + \text{Observed} \cdot \ln(\text{Expected} \cdot a \cdot (1 + bd)). \quad (4)$$

This log-likelihood ignores terms that are constant for the data set (i.e., do not depend on the values of the parameters). The maximum total log-likelihood (summed over each exposure group) was obtained and retained for future computations, as HMLLs or HMLLp, corresponding to the unconstrained human log-likelihood for the standard and PBK metrics, respectively.

2.6. Human–animal comparison of chloroprene risk estimates

The current method was developed to test the null hypotheses that certain dose metrics would provide comparable risk estimates across species, specifically mice and humans. The approach was designed to determine if one or more of the selected dose metrics was consistent with the hypothesis that there was a common risk level (across species) associated with a dose or exposure pattern of interest. The alternative hypothesis, for a given dose metric, was that the risk at the dose of interest was not the same across species.

Preliminary analyses had suggested that the benchmark dose at the extra risk level of 0.10 (BMD10) from the multistage dose–response model was just slightly less than 1 ppm, so this air concentration was selected as a reasonable concentration for comparison of risk estimates across species. For the PBK metric comparison, a value of 0.00352 l mole of CD metabolized/g-lung/day was selected as the internal dose metric of interest as that was the value estimated with model simulations conducted at either 1 ppm via an occupational exposure scenario or with the adjusted continuous exposure equivalent of 0.33 ppm.

For the ppm metric (the standard metric), a single maximized log-likelihood was determined, the unconstrained animal maximum log-likelihood for the standard metric (AMLLs) (Fig. 3). For the PBK metric, the maximum log-likelihood (AMLLp) was computed in exactly the same manner, but using the PBK metric values as the dose inputs (Table 1). Correspondingly, calculation of human relative risks was conducted by fitting the relative risk model (Eq. (3)) to the epidemiology data to define the dose–response relationship using both the standard metric (with maximum likelihood HMLLs) and the PBK metric (yielding HMLLp). Using the animal and human log-likelihood estimates, unconstrained joint log-likelihoods of observing both the animal bioassay results and the epidemiological results were computed. The joint log-likelihoods were defined as "Unconstrained" meaning that the human and animal results were computed independently of one another. The computed unconstrained joint log-likelihoods (UMLLs and UMLLp) were determined based on the animal and human maximized log-likelihoods:

$$\text{UMLLs} = \text{AMLLs} + \text{HMLLs} \quad (5)$$

$$\text{UMLLp} = \text{AMLLp} + \text{HMLLp} \quad (6)$$

i.e., the metric-specific summation of the corresponding animal and human maximized log-likelihoods.

Constrained log-likelihoods were also calculated based on the null hypothesis that the animal bioassay data and the epidemiology data would provide the same estimate of risk at the dose of interest (1 ppm or 0.00352 l mole of CD metabolized/g-lung/day, depending on the metric under consideration). A joint log-likelihood for the combined human and animal results was calculated, under the assumption of equal risks at the dose of interest. If this constrained joint log-likelihood was sufficiently close to (by a formal statistical test) the unconstrained joint log-likelihood, then the null hypothesis of equal risks at those dose values was accepted.

The constrained maximum likelihood of interest was computed by examining values of b in the relative risk model (Eq. (3)), within a range of b values extending from 0 to an upper limit sufficient (by visual inspection) to guarantee that the maximum joint constrained log-likelihood was attained. For a selected value of b, the value of a in Eq. (3) was derived that maximized the human log-likelihood. In addition, for any selected value of b, a lifetime extra risk was calculated using the life table method used by EPA and others (Federal Register, 2004; USEPA, 2002, 2011) (Appendix A). The reference population for the life table calculations was the entire US population with rates from 2008 for all causes and respiratory system cancers (CDC, 2011). Risk was computed up through age 85. The lifetime human extra risk (HER) for a selected constant exposure level (dose-of-interest, or DOI) was computed using the life table approach with the various estimates of b; it was referred to as the HER(DOI).

Given the HER(DOI) value defined above, the multistage model was fit to the animal data with an added constraint, i.e., that the animal extra risk at the DOI, AER(DOI), equals the HER(DOI). The source code for the BMDS multistage model was modified (code supplied by the authors on request) to allow for such constrained optimization; it is not possible to do it with the BMDS models as they are distributed. The modification automates the following calculations. If AER(DOI) is set equal to HER(DOI), then the multistage fit to the animal data can be maximized under that constraint:

$$\begin{aligned} \text{HER}(\text{DOI}) = \text{AER}(\text{DOI}) &= [P(\text{DOI}) - P(0)]/[1 - P(0)] \\ &= 1 - e^{-(q_1 \text{DOI} + q_2 \text{DOI}^2)} \end{aligned} \quad (7)$$

where the second equality follows from the form of the multistage model equation (Eq. (2)). Solving for q_1, results in the following equation.

$$q_1 = -\ln(1 - \text{AER}(\text{DOI})) - q_2 \text{DOI}^2)/\text{DOI} \quad (8)$$

Consequently, when AER(DOI) is fixed at a value, HER(DOI), the optimization for estimating the maximum (constrained) likelihood

[2] Even though the individual data for this cohort were available to the authors, we have used the summary data in order to demonstrate how this approach can be implemented with data that are commonly available when using epidemiological study reports for risk assessment. If we had used the individual data, we could, for example, have used a Cox proportional hazards model to better control for other variables, like age.

from the multistage model can be accomplished by varying q_0 and q_2. (i.e., all the parameters other than q_1) and then computing q_1 as shown. For the current investigation, a 2nd degree multistage model was the highest polynomial degree needed. The same assumptions would apply for a polynomial degree greater than 2.

The two log-likelihood components, human and mouse, were then summed:

$$CMLLx(b) = HMLLx(b) + AMLLx(b); \quad (9)$$

indicating the dependence on the choice of b. The value of "x" in Eq. (9) was either s (for the standard, ppm metric) or p (for the PBK metric), just as for the unconstrained likelihood calculations. The full range of allowable b values was examined to determine a maximum for CMLLx(b); that maximum was the maximum constrained log-likelihood, CMLLx.

A likelihood ratio test was used to test the null hypothesis that the constraint of equal risks at DOI was true. The test statistics were:

$$2 \cdot (UMLLx - CMLLx) \quad (10)$$

(twice the differences in the log-likelihoods, x = s or p). There is one degree of freedom associated with the chi-squared distribution that approximates the distribution of those test statistics (Eq. (8) demonstrates there is one less parameter to be estimated, i.e., q_1, when the constraint of HER(DOI) = AER(DOI) is in effect, that is, when the null hypothesis is true). Larger differences in the maximized likelihoods yield larger values of the test statistic and therefore smaller p-values (i.e., probabilities of being in the tail of the chi-squared distribution to the right of the test statistic value). Small p-values (less than 0.05) were indicative of the null hypothesis being false.

2.7. Uncertainty analyses

An uncertainty analysis was conducted to evaluate the potential impact of the assignment of CD exposure concentrations (ppm) to the workers in the Louisville cohort. Esmen et al. (2007a) assigned nominal exposure levels to the members of the Louisville cohort, depending upon job class and calendar year. The uncertainty in the nominal levels was considered using "subtitles" for jobs within job class, the type of rotation among workers within those subtitles, and the deciles of the varying exposure levels associated with those subtitles. A Monte Carlo analysis was conducted, generating 3000 simulated human data sets, to evaluate the impact of exposure uncertainty. Each simulated human data set assigned different ppm exposure levels to each worker's work history, consistent with exposure uncertainty distributions defined in the Supplemental material; a detailed description of the approach used in the Monte Carlo for the assigning of exposures concentrations to the workers is provided in that Supplemental material.

Given the rules specified in the Supplemental material, 1500 alternative (simulated) exposure histories for the cohort members were generated and run through the OCMAP program (Marsh et al., 1998). The output of each of those runs was a set of dose–response data analogous to those shown in Table 2. The cut points for defining the exposure groups were the same as used in the original analysis (Marsh et al., 2007b) (second column of Table 2).

When considering the PBK metric for humans, the above procedure was used to generate another set of 1500 simulated data sets, but an additional step was included to represent the uncertainty between the ppm exposure level and the PBK dose metric value. That additional step utilized the posterior distributions of the PBK model parameters derived by Yang et al. (2012). Following the assignment of each ppm exposure level as described in the Supplemental material, a PBK metric value was generated by sampling from a lognormal distribution with (natural scale) mean and coefficient of variation equal to,

Table 3
Heuristic for comparing models via Bayesian Information Criteria (BIC) values.

DBIC[a]	Strength of evidence
< −10	Very strong evidence for model i
−10 to −6	Strong evidence for model i
−6 to −2	Positive evidence for model i
−2 to 2	Not much evidence either way
2 to 6	Positive evidence against model i
6 to 10	Strong evidence against model i
>10	Very strong evidence against model i

[a] DBIC = BIC(i) − BIC(j), where BIC(k) is the BIC associated with model k. Based on the categorization shown in Kass and Raftery (1995).

$$\begin{aligned} l &= 0.00373 \text{ ppm} \\ CV &= 0.74; \end{aligned} \quad (11)$$

respectively. Those values for l and coefficient of variation (CV) (the log-scale variance equals $\ln[1 + CV^2]$) were selected based on the following observations. The posterior distributions of the PBK model parameters (Yang et al., 2012) were sampled 500 times each for five exposure concentrations ranging from 0.016 to 160 ppm (by factors of 10)[3] and the associated PBK metric values (for the occupational exposure scenario) were computed for each sampling. As discussed elsewhere, the human ppm-to-PBK metric conversion is linear (for this range of ppm exposure levels); the factor of 0.00373 was associated with the average of the 2500 generated PBK metric values. Similarly, a CV of 0.74 was consistent with the variation observed across all those generated PBK metric values (conditional on the value of the mean).

The cut points on cumulative PBK metric values used to assign person years of observation to four exposure groups were those shown in Table 2 (second column) multiplied by 0.00352 (the conversion factor obtained when using PBK model parameter values equal to the means of each posterior distribution).

For each of the 3000 simulated data sets, the unconstrained and constrained maximization of the log-likelihoods was completed just as described in Section 2.5 above. For interpretation of the results of the uncertainty analysis the Bayesian Information Criteria (BICs) were used to evaluate the strength of the evidence for or against any given model. The BIC is defined as,

$$BIC = -2 \cdot MLL + \ln(n) \cdot parms; \quad (12)$$

where MLL, is the maximized log-likelihood, n is the number of observations, and parms is the number of parameters in the model. For the joint log-likelihoods (across mouse and human data sets) that we are analyzing here, n = 8 (four dose groups each for the mice and humans); parms = 5 for the unconstrained model (mouse and human data fit separately and independently) and parms = 4 for the constrained model (see Eq. (7) and associated text for a discussion of the reduction in the number of parameters under the constraint of equal risk at the DOI).

Lower values of the BIC indicate a better model. The BIC (like other information criteria) "rewards" a model for better fit (greater log-likelihood) but "penalizes" a model that uses more parameters to achieve a better fit. Put another way, the BIC rewards fit and parsimony.

A model comparison heuristic was introduced by Jeffreys (1961) and refined by Kass and Raftery (1995) (Table 3); it provides a categorization of the strength of the evidence for or against a given model, relative to another model. In our case, DBIC was defined with the unconstrained model as the referent, DBIC = BIC

[3] These exposure levels were those reported in Esmen et al. (2007a,b) as the nominal chloroprene levels for their exposure classes (see their Table 2).

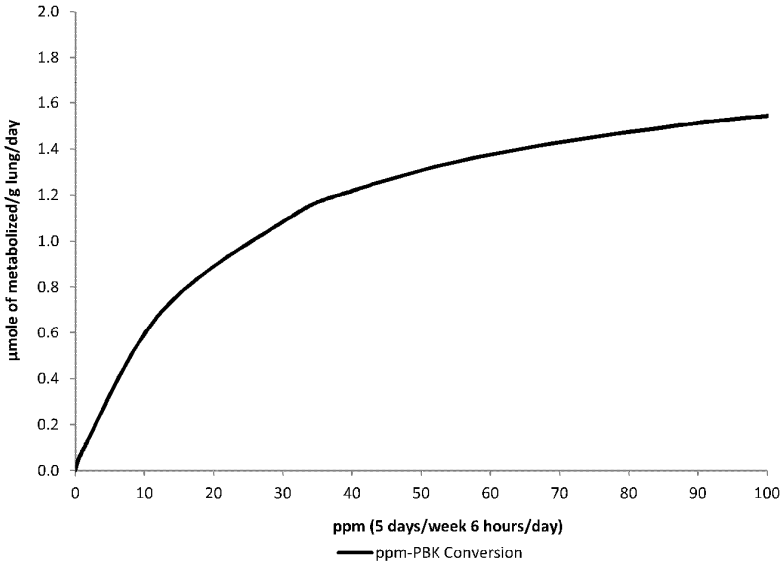

Fig. 2. Relationship between experimental exposure levels and PBK metric values; female mice.

(constrained) − BIC (unconstrained). Therefore, negative values of the DBIC favor the constrained model; positive values favor the unconstrained model. The results of the uncertainty analysis were summarized by tabulating the number of iterations of the simulations for which the constrained model falls in each of the evidence categories.

3. Results

The animal data set (Table 1) was not well described by the multistage model, when the doses were expressed in terms of the ppm exposure levels. The p-value for goodness-of-fit was 0.0046, a p-value indicating inadequate fit of the model to the data (p-values of greater than 0.10 are considered an adequate fit (USEPA, 2005)). The use of the PBK dose metric resulted in an adequate fit of the multistage model to the animal data (p-value = 0.44). Because of the saturation of metabolism in the lungs of female mice within the range of the experimental exposures (Fig. 2), the use of the internal PBK dose metric better correlated with the lung tumor incidence in the mouse than the external ppm dose metric. The PBK transformation was successful with respect to making differences in delivered dose accord with differences in response rates, when a multistage model represents the underlying carcinogenic process for the selected respiratory system cancer response.

The unconstrained, maximized log-likelihoods for the animal models were AMLLs = − 105.758 (for the standard, ppm metric) and AMLLp = − 101.049 (when using the PBK metric). The increase in the log-likelihood with use of the PBK metric is also indicative of a better fit, relative to use of the ppm exposure levels.

The human dose–response data (Table 2), were best fit by a relative risk model (Eq. (3)) with a slope (b) of zero and a = 0.74. The fact that b = 0 is consistent with the absence of a dose–response relationship between cumulative exposure and respiratory system cancer deaths in those workers.[4] This was true whether or not the dose was expressed in terms of ppm-years or (lmole/g lung/day)-years, at least partially because the PBK transformation in humans was linear for the relatively low exposure levels experienced by this cohort (Fig. 3). The maximized log-likelihood for the relative risk model with 0 slope was HMLLs = HMLLp = 849.396 (regardless of the dose metric used).

Therefore, the "base case," unconstrained maximized combined log-likelihoods were,

UMLLs ¼ 743:638 ð13Þ
UMLLp ¼ 748:347

for the ppm exposure metric and for the PBK metric, respectively (Table 4).

3.1. Human–animal comparison of chloroprene risk estimates

The constrained optimization considered the animal and human data simultaneously, and maximized the sum of the animal and human log-likelihoods subject to one constraint, that the extra risk for the two fitted models be the same at the DOI. For the ppm exposure metric, the maximum constrained log-likelihood was attained when the relative risk slope was b = 0.0017 (per ppm-year). For that slope estimate, HMLLs(b) = 848.345, AMLLs(b) = − 118.063 and therefore CMLLs = 730.282 (Table 4). The comparison of the constrained maximum log-likelihood to the unconstrained maximum log-likelihood (UMLLs = 743.638) indicates a statistically significant difference (p-value = 2 × 10^{-7}). This indicates that the animal- and human-based risks at 1 ppm are not the same (i.e., rejection of the null hypothesis). For the PBK metric, the DOI was set to 0.00352 lmole of CD metabolized/g lung/day, the PBK dose-metric that corresponds to an occupational exposure of 1 ppm. Under the constraint that the animal extra risk was the same as the human extra risk at that dose, the maximum constrained log-likelihood was attained when the relative risk slope was b = 0.125 (per (lmole/g lung/day) – years), and HMLLp(b) = 848.676, AMLLp(b) = − 101.254, and therefore CMLLp = 747.422.

The PBK metric provides consistent cross-species low-dose risk estimates (the p-value for the test of the null hypothesis equals 0.17). The null hypothesis of equal risk at the PBK dose of 0.00352 lmole/g lung/day would not be rejected at the typical

[4] For the relative risk model, the slope was constrained to be non-negative. No evaluation was conducted to determine if negative values for the slope were better than zero. It was considered implausible that chloroprene exposure would reduce respiratory cancer risk.

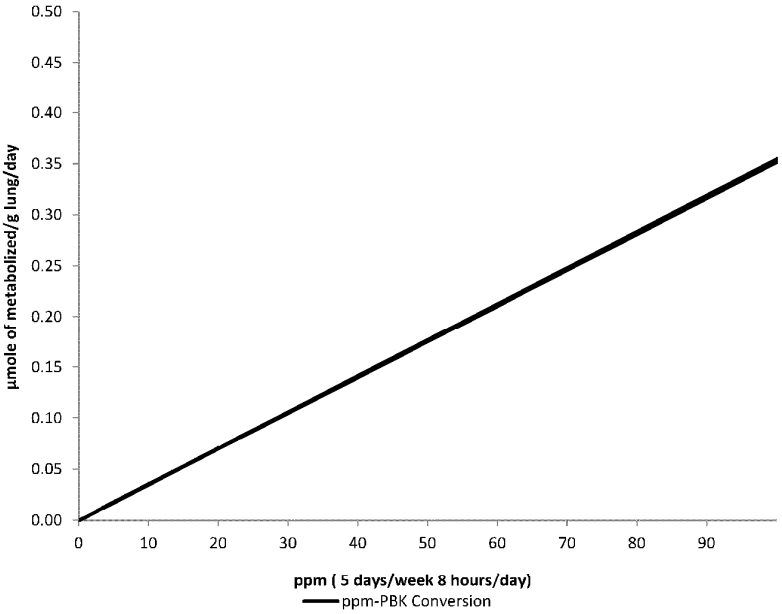

Fig. 3. Relationship between occupational exposure levels and PBK metric values; humans.

0.05 level of significance. Not only did the PBK transformation of doses result in a substantially improved model fit to the animal data, it also reconciled cross-species predictions of risk estimates for low doses.

Table 4
Unconstrained and constrained maximized log-likelihoods.

Dose-metric	Animal	Human	Combined
Unconstrained			
ppm metric	└ 105.758	849.396	743.638
PBK metric	└ 101.049	849.396	748.347
Constrained			
ppm metric	└ 118.063	848.345	730.282
PBK metric	└ 101.254	848.676	747.422

Naturally, the unconstrained fit to the animal data provided the best fit. Although the constrained fit to the animal data (where the animal risk at the DOI was constrained to equal the human risk at the DOI) was not as good as the unconstrained fit, the predicted probabilities of response were still well within the (1 SE) error bars associated with the observed response rates (Fig. 4). Importantly, the constrained curve had a less steep slope at low doses, which conforms better to the (at most) shallow slope for the human dose–response. The achievement of a shallow low-dose slope with enough curvature to match the observations at the higher

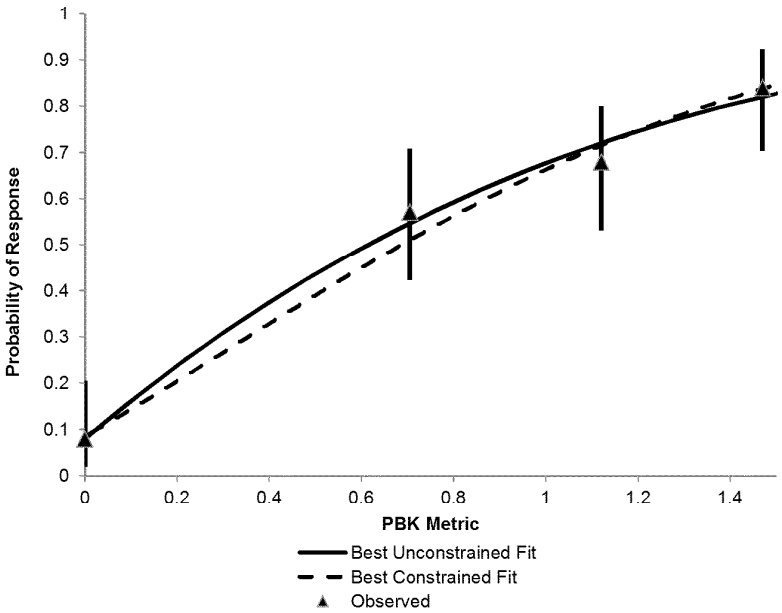

Fig. 4. Comparison of best unconstrained and constrained fits to animal data.

Table 5
Evidence for and against the constrained model, by exposure metric.[b]

DBIC[a]	Strength of evidence	No. simulated cohort data sets in each category	
		ppm metric	PBK metric
< −10	Very strong evidence for constrained model	0	736
−10 to −6	Strong evidence for constrained model	1	284
−6 to −2	Positive evidence for constrained model	16	236
−2 to 2	Not much evidence either way	46	162
2 to 6	Positive evidence against constrained model	131	63
6 to 10	Strong evidence against constrained model	259	13
>10	Very strong evidence against constrained model	1047	6

[a] DBIC = BIC(constrained) − BIC(unconstrained).
[b] Each simulated cohort data set was subject to constrained and unconstrained maximum likelihood estimation. The final two columns shows the number (out of 1500) of those data sets that had different degrees of support for or against the constrained model, depending on the choice of exposure metric.

experimental exposure levels is what allows for a consistent risk estimate at the DOI.

3.2. Uncertainty analyses

Uncertainty in estimated human exposures had an interesting effect on the comparison of the constrained and unconstrained models (Table 5). For the models applied to the ppm metric, exposure uncertainty implied a range of estimates that predominantly did not support the constrained model; all but 63 (of 1500) simulated exposure runs demonstrated evidence against the constrained model and, therefore, against the hypothesis that mice and humans have equal risk at 1 ppm (when risks were equilibrated on the basis of ppm exposure levels). When the PBK metric was used, there was a notable shift to values that favor the constrained model. A total of 1256 runs demonstrated evidence for the constrained model (nearly half were consistent with very strong evidence in favor of the constrained model and, therefore, for the equality of animal and human risks at low doses). The ability to eliminate one parameter in the optimization was of key importance, especially when the log-likelihoods for the constrained and the unconstrained models were similar. The DBIC for the base case (no uncertainty) constrained model using the PBK metric was −0.23, i.e., little or no evidence for or against it relative to the unconstrained model. This result is consistent with the failure to reject the null hypothesis of no difference in risk across species at the PBK dose of interest.

4. Discussion and conclusions

The analysis described here presents a new method to compare and test risk predictions across species for lifetime extra cancer risk. It requires that specific methods be applied as appropriate to the type of data available, but all having the goal of predicting lifetime extra cancer risk. Thus, for the epidemiological data, relative risk Poisson modeling linked to life-table calculations yields the necessary risk estimates. For the animal bioassay data, multistage modeling is applied. Those two sides of the analysis were subject to a formal statistical evaluation that addressed hypotheses of interest using likelihood procedures.

This approach allows for reproducible and consistent comparisons of experimental and/or observational data that are commonly used for risk assessment purposes. In the specific case of CD, the results of applying this approach indicate that external, concentration-based estimates of exposure to CD are not the appropriate dose metric for estimating comparable risk estimates across species. Even when accounting for one of the largest uncertainties associated with the use of epidemiological data for dose–response assessment, i.e., reconstructing occupational human exposure levels, there was little or no statistical support for the hypothesis that human and animal low-dose risks are equivalent when exposure was expressed in terms of ppm air concentration. Conversely, the use of the PBK metric, daily amount of CD metabolized at the target per gram of tissue, in the dose–response models provided better fit of the models to the data due to the ability of the PBK metric to account for the cross-species metabolic differences. It also resulted in comparable risk estimates across species at the dose of interest, and more generally, at all doses less than or equal to the dose of interest.

The evaluation of the animal and human data using the PBK metric provided cancer slope factors between 2.9×10^{-5} and 1.4×10^{-2} per ppm, with the maximum-likelihood estimate of 6.7×10^{-3} per ppm. The human equivalent cancer slope factor estimated based on the incidence of lung tumors in female mice (the most sensitive sex and species) reported in the EPA Toxicological Review (2010) is 6.5×10^{-1} per ppm (adjusted for exposure 6/24 h and 5/7 days). This slope factor is approximately 100 times greater than the maximum-likelihood estimate determined with the current approach.

While the current adjustment for pharmacokinetic differences across species results in comparable risk estimates, there are

Table 6
Evaluation of the presence of pharmacodynamic differences across species.

Relative pharmacodynamic sensitivity	Mouse PBK metric value (lmole of CD metabolized/g lung/day)	Mouse metric/ human metric	Test of equality of risks at the specified PBK doses (p-value)[a]
Humans more sensitive	0.0845	24	0.001
	0.0282	8	0.029
	0.00845	2.4	0.056
Humans equally sensitive	0.00352	1	0.17
Humans less sensitive	0.00282	0.8	0.22
	0.000845	0.24	0.54

[a] P-values are from the test of various null hypotheses, i.e., that the risk at the specified mouse metric values is equal to the risk at the human PBK metric value of 0.00352 lmole/g lung/day (the constrained maximum likelihood calculations). The alternative hypotheses are that there is no such constraint; the mouse and human models are independent so do not necessarily predict equivalent risks at the specified doses.

additional factors that could be considered to further refine the evaluation. These could include species-specific differences in detoxification and pharmacodynamics.

In the case of CD, the data are not currently available to estimate or model the magnitude of species differences in such additional factors. However, the current analysis approach provides evidence that, if and when such data become available they will demonstrate that humans are equally or less sensitive, but not more sensitive than mice, at the low levels of CD exposure investigated. That "working hypothesis" results from the analysis results shown in Table 6. If one assumes that risk is equal when the human PBK metric value is 0.00352 Ì mole CD metabolized/g-lung/day and the mouse metric value is at different levels (greater or less than 0.00352), equivalence of risk was only supported (having p-values greater than 0.05) when the proposed equivalent-risk mouse dose was less than or equal to about 2.4 times the human dose of 0.00352. The working hypothesis of lower human low-dose risk still remains to be tested formally with data specifically obtained and appropriate for that purpose. Until then, the results of the current analyses suggest that humans are equally or less sensitive than mice to equivalent low-dose CD exposures.

Conflict of interest

B.C. Allen: Sub-contract to ENVIRON International Corporation and The Hamner Institutes for Health Sciences. C. Van Landingham: Contract to ENVIRON International Corporation. Y. Yang: Personal fees from International Institute of Synthetic Rubber Produces, Inc. (IISRP). A.O. Youk: Grants from International Institute of Synthetic Rubber Produces, Inc. (IISRP) and personal fees from DuPont Chemical Company. G.M. Marsh: Grants from International Institute of Synthetic Rubber Produces, Inc. (IISRP), personal fees from DuPont Chemical Company. N. Esmen: Nothing to disclose. P.R. Gentry: Contract to ENVIRON International Corporation. H.J. Clewell III: Personal fees from International Institute of Synthetic Rubber Produces, Inc. (IISRP). M.W. Himmelstein: Nothing to disclose.

Acknowledgments

This work was conducted under contracts awarded to The Hamner Institutes for Health Sciences, ENVIRON International, the University of Pittsburgh and the University of Illinois at Chicago by International Institute of Synthetic Rubber Produces, Inc. (IISRP).

Appendix A. Formulae for calculating extra risk using a life-table method

The probability of disease occurrence (incidence or mortality) between ages x_1 and x_2 may be expressed as:

$$p(0) = \int_{x_1}^{x_2} h(x)S(x)dx \qquad (A1)$$

where S(x) is the probability of survival to age x given survival to age x_1 and h(x) is the instantaneous hazard of disease occurrence at age x. This integral can be approximated by a sum:

$$p(0) = \sum_{i=1} p(i)S(i) \qquad (A2)$$

where the age interval $[x_1, x_2]$ has been divided into n subintervals with the ith subinterval having width D(i), i = 1 „ . . . , n, p(i), representing the probability of disease occurrence in the ith age interval, is calculated as:

$$p(i) = q_c(i)D(i); \qquad (A3)$$

and S(i), representing the probability of surviving to the beginning of the ith age interval given survival to age x_1, is calculated as S(1) = 1 and:

$$S(i) = \prod_{j=1} \exp\left(-q_a(j)D(j)\right) = \exp\left[-\sum_{j=1} q_a(j)D(j)\right]; \quad i > 1 \qquad (A4)$$

where $q_c(i)$ and $q_a(i)$ are the cause-specific rate of occurrence and all-cause death rates for the ith age interval obtained from standard rate tables. An alternative to (Eq. (A4)) is given by:

$$S(i) = \prod_{j=1} \left[1 - q_a(j)D(j)\right]; \quad i > 1; \qquad (A5)$$

which encompasses slightly different interpretations of the standard rates. These 2 expressions generally agree closely.

If the subintervals correspond to individual years, (Eqs. (A2) and (A4)) take on the simplified forms:

$$p(0) = \sum_{i=x_1} q_c(i)S(i); \qquad (A6)$$

and:

$$S(i) = \prod_{j=x_1} \exp\left(-q_a(j)\right) = \exp\left[-\sum_{j=x_1} q_a(j)\right] \qquad (A7)$$

Once the background rates q_c and q_a are selected, these equations completely determine p(0). These same formulae are used to calculate the probability of response, p(D), from a particular exposure pattern, D, by replacing the rates q_c and q_a by the appropriate modification that accounts for the model-predicted effect of exposure on these rates. The appropriate modifications depend upon the form of the dose–response model estimated from the epidemiologic data, and the assumed exposure pattern. If the dose–response model predicts relative risk as a function of some exposure metric, then:

$$q_c(i) \text{ is replaced by } q_c(i)R(i) \qquad (A8)$$

and:

$$q_a(i) \text{ is replaced by } q_a(i) - q_c(i)D(i) + R(i)q_c(i)D(i) = q_a(i)D(i) + q_c(i)D(i)R(i) - 1]; \qquad (A9)$$

where R(i) is the relative risk predicted by the dose-response model, i.e., R(i) = 1 + b * D(i), where D(i) is the cumulative dose at age i from exposure pattern D. The latter replacement involves subtracting from the total death rate the background death rate from the disease of interest, and adding back this contribution adjusted by the effect of exposure.

Once p(0) and p(D) have been calculated, the extra risk from exposure pattern D is computed as:

$$[p(D) - p(0)]/[1 - p(0)] \qquad (A10)$$

This extra risk is what will be compared with the animal-based extra risk estimate.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.yrtph.2014.07.001.

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Toxicology in Vitro 26 (2012) 1047–1055

Contents lists available at SciVerse ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Kinetic modeling of b-chloroprene metabolism: Probabilistic in vitro–in vivo extrapolation of metabolism in the lung, liver and kidneys of mice, rats and humans

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article info

Article history:
Received 25 January 2012
Accepted 4 April 2012
Available online 19 April 2012

Keywords:
In vitro
Kinetic modeling
Bayesian
PBPK
IVIVE

abstract

b-Chloroprene (chloroprene) is carcinogenic in inhalation bioassays with B6C3F1 mice and Fischer rats, but the potential effects in humans have not been adequately characterized. In order to provide a better basis for evaluating chloroprene exposures and potential effects in humans, we have explored species and tissue differences in chloroprene metabolism. This study implemented an in vitro–in vivo extrapolation (IVIVE) approach to parameterize a physiologically based pharmacokinetic (PBPK) model for chloroprene and evaluate the influence of species and gender differences in metabolism on target tissue dosimetry. Chloroprene metabolism was determined in vitro using liver, lung and kidney microsomes from male or female mice, rats, and humans. A two compartment PK model was used to estimate metabolism parameters for chloroprene in an in vitro closed vial system, which were then extrapolated to the whole body PBPK model. Two different strategies were used to estimate parameters for the oxidative metabolism of chloroprene: a deterministic point-estimation using the Nelder-Mead nonlinear optimization algorithm and probabilistic Bayesian analysis using the Markov Chain Monte Carlo technique. Target tissue dosimetry (average amount of chloroprene metabolized in lung per day) was simulated with the PBPK model using the in vitro-based metabolism parameters. The model-predicted target tissue dosimetry, as a surrogate for a risk estimate, was similar between the two approaches; however, the latter approach provided a measure of uncertainty in the metabolism parameters and the opportunity to evaluate the impact of that uncertainty on predicted risk estimates.

1. Introduction

b-Chloroprene (chloroprene, 2-chloro-1,3-butadiene, CAS 126–99-8) is a volatile colorless liquid used to manufacture polychloroprene, a synthetic rubber (Lynch, 2001a). Occupational exposure can occur during monomer synthesis, shipping, and polymerization processes, and inhalation is the only significant route of exposure (Lynch, 2001b). The health effects in humans have focused on the potential carcinogenicity of chloroprene in the liver, lung and lymphohematopoietic systems (reviewed by Bukowski, 2009). Although epidemiological findings do not support a substantial link between chloroprene exposure and increased cancer mortality (Marsh et al., 2007), it is still important to understand species differences.

Extensive animal studies have been performed to understand possible adverse health effects of chloroprene in humans including acute, sub-chronic, and chronic toxicity studies (Melnick and Sills, 2001; Valentine and Himmelstein, 2001; Pagan, 2007). The most toxicologically significant finding was chloroprene-induced tumorigenicity in F344/N rats and B6C3F1 mice exposed to 680 ppm for 2 years (Melnick et al., 1996, 1999; NTP, 1998). Tumors in Fischer rats included the lung, oral cavity, thyroid gland, kidney, and mammary gland. Mouse tumors were in the lung, circulatory system, Harderian gland, forestomach, kidney, mammary gland, skin, mesentery, Zymbal gland, and liver. In contrast, no tumors occurred in Syrian hamsters and only a weak response in mammary tissue in female Wistar rats (Trochimowicz et al., 1998) indicating species and gender differences in tumorigenesis in rodents.

Chloroprene is oxidized by cytochrome P450 enzymes (Cottrell et al., 2001; Himmelstein et al., 2001b). One reactive intermediate formed is the epoxide (1-chloroethenyl) oxirane which was mutagenic in the Ames assay, but not clastogenic at cytotoxic concentrations in vitro (Himmelstein et al., 2001a). This epoxide also shows reactivity with DNA in vitro and is a potential cross-linking agent (Munter et al., 2002; Wadugu et al., 2010). The reactive metabolites of chloroprene are likely to contribute to the tumorigenicity of chloroprene seen in animal studies. Given the important role of metabolic activation for toxicity, it is important to understand chloroprene metabolism to assess its potential health effects. To this end, a physiologically based pharmacokinetic (PBPK) model

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http://dx.doi.org/10.1016/j.tiv.2012.04.004

was developed for chloroprene based on in vitro metabolism data. Previous PBPK models for chloroprene in male rodents and humans (Himmelstein et al., 2004a, b) suggested significant differences in chloroprene metabolism among liver and lung and among different species. Intrinsic clearance of chloroprene metabolism in hepatic microsomes was two fold higher in mouse compared to human, while clearance in lung microsomes was forty times higher in mouse than either rat or human. With the application of PBPK modeling, the species differences in metabolism (amount chloroprene metabolized per gram lung tissue) were shown to be the underlying mechanism for the difference in lung tumor incidence among different species (Himmelstein et al., 2004b).

Here we extend the chloroprene PBPK model using additional data for chloroprene metabolism from different species and genders. The models evaluated the role of metabolism differences in species- and sex-dependent tissue dose metrics (a potential marker for tumorigenesis). A key objective of this effort was to develop a probabilistic parameter estimation approach; so that the impact of uncertainty in the metabolic parameter estimates on risk predictions can be as illustrated in Fig. 1. While previous studies used deterministic approaches to estimate metabolism parameters, we estimated these parameter values by two different methods: deterministic point-estimation and a probabilistic (Bayesian) approach.

2. Materials and methods

2.1. In vitro microsomal experiments

2.1.1. Chemicals

b-Chloroprene (>99%) containing phenothiazine and N-nitrosodiphenylamine inhibitors was supplied by DuPont Performance Elastomers,LLC (LaPlace, LA). The inhibitors were removed as previously described (Himmelstein et al., 2001b). The purified chloroprene was stable at <ffi70 ffC under nitrogen headspace atmosphere. For metabolism experiments, vapor concentrations were prepared by adding the liquid test substance to Tedlar® bags (SKC Inc., Eighty Four, Pennsylvania, USA) containing a known volume of room air. Further gas phase dilutions were made for calibration or exposure purposes. Gas tight syringes were used for the gas transfers.

2.1.2. Source of microsomes and cytosol

Fischer rat (F344/DuCrl) and mice (B6C3F1/Crl) were received from Charles River Laboratories, Inc., Raleigh, North Carolina. The species and strains were selected to match those used for inhalation toxicity testing by the National Toxicology Program (NTP, 1998). The animals were acclimated for at least 7 days prior to use. A total of 15 female rats and 50 female mice were used for preparation of the liver and lung microsomes. A total of 15 rats/sex and 30 female mice/sex were used for preparation of kidney microsomes. Human kidney microsomes were purchased from Xenotech (H0610.R, Lot No. 0810236, Lenexa, Kansas, USA). Microsomes were prepared by differential centrifugation and pooled as described by Himmelstein et al. (2004a). The use of pooled tissue microsomes mitigates issues of inter-animal biological variability, yet supports the analysis on species and gender differences. Further details on the microsomal preparation are given in the Supplement data A.1

2.1.3. Microsomal oxidation of chloroprene

The time course of total chloroprene disappearance was measured in three tissues: liver and lung microsomes for female rodent; kidney microsomes of rodents for both genders and human kidney microsomes. Data on the (1-chloroethenyl) oxirane formation was not collected in the current experiments because of the focus on total chloroprene metabolism as a dosimetric for dose–response modeling (Himmelstein et al., 2004b). After pre-incubation (37 ffC for 5 min), an equal volume of vial headspace was removed from the vial and replaced with known concentrations of chloroprene vapor. The vial was equilibrated for approximately 10 min and reactions were started by the addition of microsomal protein and $NADP^+$ (0.53 mM). Microsomal protein concentrations were established from previous work (liver and lung) or experimentally for kidney microsomes. Definitive experiments used protein concentrations that ranged from 1–3 mg/mL. Control incubations were performed without $NADP^+$ or with $NADP^+$ and heat-inactivated microsomes. Samples (200 lL) were injected on the GC using a robotic x-y-z programmable multipurpose sampler (MPS2, Gerstel US, Baltimore, Maryland, USA) and were analyzed at 12 min intervals for up to 1 h.

2.2. In Vitro Kinetic Model Description

A 2-compartment PK model modified from Himmelstein et al. (2004a) was used to describe the time-concentration measurements of chloroprene in the headspace in the closed vial system. The microsomal oxidation of chloroprene in tissues (liver, lung and kidney) was by saturable kinetics, with the exception of rat and human lung where a first-order process was used. In addition to microsomal metabolism, the current model included the loss of chloroprene from the headspace to describe the decline of headspace concentration of chloroprene observed in the control dataset. The background loss rates were modeled as a first order process. Estimates of the first order background loss rates were based on eight sets of control data (the complete female data set plus the male kidney dataset). The in vitro experimental background loss rate was assumed to be independent of gender, tissue, and dose. The same PK model was used to estimate the background loss rate by setting the parameter values for the microsomal process to zero. To estimate the gender-specific variability of the kinetic parameters, male tissue

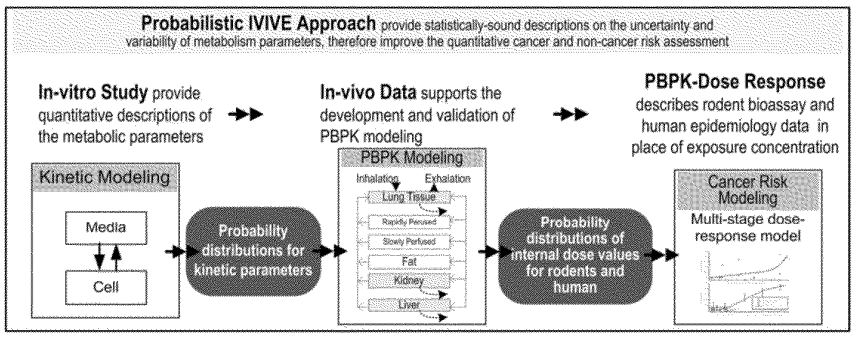

Fig. 1. Illustration of probabilistic approach.

data from Himmelstein et al. (2004a) were re-evaluated using the updated PK model. For a more detailed description of the male dataset and the 2-compartment model, see Himmelstein et al. (2004a).

2.3. Kinetic parameter deterministic (point) estimation

All model parameters were optimized with ACSL-Optimize (version 11.8.4, AEgis, Technologies Group, Inc, Huntsville, Alabama, USA), using the Nelder-Mead method with a relative error minimization-based, log-likelihood function.

2.4. Kinetic Parameter Probabilistic (Bayesian) Analysis

2.4.1. Two-level hierarchical Bayesian model for rat and mice

A two-level hierarchical Bayesian model was used to estimate the gender-variability of the in vitro metabolic parameters. This approach was hierarchical in the sense that the uncertain population level (species) parameters at the top level define the variability of the lower-level (gender) parameter values. Inter-gender variability for a given microsomal activity parameter (in log-scale) was described by a normal distribution with population mean M and standard deviation S. The prior distribution of M was uniform (Table 1). The same log-uniform distributions were used for all model parameters (V_{max}, k_m and V_{max}/k_m) for all animal species, tissues, and doses. The log-uniform distribution [ffi10, 5] was broad enough to encompass the actual distributions of the metabolic parameters. The initial mean values were determined from the point estimation results in Himmelstein et al. (2004a), and two preliminary MCMC analyses. Before a fixed log-uniform distribution [ffi10, 5] was selected, two uniform distributions were tested for microsomal activity parameters; one [1e-8, 500] (natural scale); and the other [ffi20, 10] (log-scale). All three priors produced the identical posterior results given the same variability and error model. The log-uniform [ffi10, 5] was chosen to reduce the computational sampling time.

Prior descriptions of gender-specific variability (S) were lognormal [0.3, 5]. Because the MCMC parameters were sampled in log-space, the estimated gender-specific variability was an equivalent description to the coefficient of variation. One additional distribution, lognormal [0.3, 1], was tested in the preliminary analysis. Given the same prior conditions on other parameters, the posterior results obtained from the alternative priors for gender-specific variability were very comparable. The broader prior (lognormal [0.3, 5]) was selected to avoid over-constraining the posterior parameters. Computational procedures for the MCMC analysis are provided in Supplemental data A.2.

2.4.2. Population-only Bayesian model for human

Gender-specific microsomal activity data were not available for human tissues. A single-level MCMC simulation was performed using the prior distributions and likelihood functions from the 2-level hierarchical model. Estimation of population-only posterior distributions reflected the combined uncertainty and variability of model parameters calculated using the mixed gender microsomal human data.

2.4.3. MCMC computation process

Nine MCMC analyses were performed for this study (control dataset for background loss rate; liver, lung, and kidney for rat and mouse, and liver and lung for human). The human kidney microsomal metabolism data was not modeled because of the failure to observe experimentally measurable chloroprene uptake. Three MCMC chains were run for each analysis. A minimum of 200,000 iterations were performed for each chain. The first 100,000 iterations initialized the Monte Carlo chain ('burn-in' period) and the remaining 100,000 iterations were used for convergence testing and data analysis.

Table 1
Prior distributions for in vitro chloroprene metabolism parameters.

Parameter application	V_{max}, k_m, V_{max}/k_m[a]	
	Distribution	Truncation
Population (exp(M))	Uniform	[4.5e-5, 150]
Gender variability (S)	Lognormal (0.3, 5)	[0.01, 10]
Individual (exp(m))[b]	Exp(Normal (M, S))	[2e-9, 2e4]

M – mean, exp(M) – exponential of mean, S – standard deviation.
[a] Units: V_{max} (lmol/h/mg), k_m (lmol/L), V_{max}/k_m (L/hr/g protein).
[b] Individual level parameter refers to gender-specific metabolic parameters in the 2-compartment in vitro PK model.

The MCMC analysis of background loss rate was executed first, prior to the other eight MCMC analyses. The derived posterior distribution of background lose rate was used as a fixed input for the MCMC analyses of chloroprene oxidation data for various tissues and species to account for the background loss of chloroprene in the headspace (in addition to removal of chloroprene during headspace sample extraction).

The method of Brooks and Gelman (1998) was used to diagnose the convergence of MCMC chains. Three MCMC chains were run for each analysis. Once the MCMC chains converged to a stationary distribution, the "converged" parts of the chains were considered representative samples from the posterior distributions (corrected scale reduction factor (CSRF) <1.2). After the chains converged, 4000 sets of the parameters were randomly sampled to represent the posterior distributions. Presentation of the results included probability frequencies, mean (exp(m)) and standard deviation (std(exp(m))) estimates of the 50th percentile central tendencies, and time course plots of chloroprene headspace concentrations with model estimates for a distribution of 50 simulated samples.

An example of the model code for one of the MCMC analyses is provided in the Supplementary data. This MCMC analysis was performed using acslX by The AEgis Technologies Group, Inc.

2.5. In vitro–in vivo extrapolation of chloroprene metabolic Constants

2.5.1. PBPK model structure

A chloroprene PBPK model was first developed by Himmelstein et al. (2004b) to describe inhalation exposure of this chemical in mice and rats. The current model structure was adapted from this multispecies PBPK model with the addition of a kidney compartment. It is a flow-limited PBPK model with six tissue compartment: lung, liver, kidney, fat, slowly and rapidly perfused tissues. As in the original model, metabolism of chloroprene was included in the liver and lung mediated by cytochrome-P450 enzymes. This oxidative pathway was saturable process except in the lung in rats and humans. For these two cases, it was a first-order process. Metabolism of chloroprene in the kidney was via a P450 mediated saturable pathway for rats, mice, and humans.

Most of the physiological and biochemical parameters used in the current PBPK model were from the original model, with some modifications. Physiological parameter values were adapted from Brown et al. (1997). Tissue-to-blood partition coefficients were calculated using the means of the experimental tissue-to-air partition values in Himmelstein et al. (2004b). The partition coefficients for rapidly perfused and slowly perfused tissue compartments were the same as those reported for the kidney and muscle, respectively. In vitro-derived gender-specific metabolism parameters were used to estimate chloroprene metabolism in liver, lung and kidney. Scaling of these in vitro parameters to the corresponding in vivo parameters followed the same steps as described in Himmelstein et al. (2004b). It was assumed that there is no-gender difference in the microsomal protein contents among different species, the

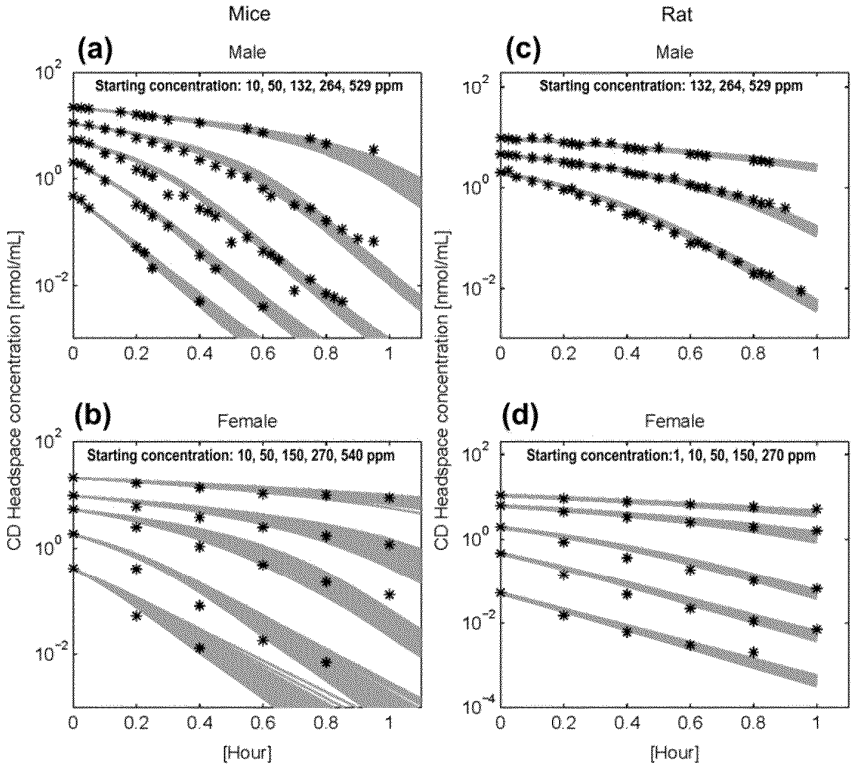

Fig. 2. Distributions of chloroprene oxidative metabolism time-course predictions versus experiment data (symbols) in liver microsomes. Chloroprene headspace concentrations were collected for various starting headspace concentrations. Simulated time course data (lines) were based on posterior distribution for parameter values reported in Table 5. Simulations represent 250 sets of model parameters randomly drawn from the posterior distributions.

values of which for the liver were 35, 49, and 56.9 mg protein/g liver for mice, rats, and humans, respectively (Himmelstein et al., 2004b). For lung and kidney microsomes, 23 and 11.5 mg protein/g was used for all animal species (Himmelstein et al., 2004b).

To quantitatively compare the effect of gender difference of the metabolism parameters on the dose–response analysis, we calculated the gender-specific internal tissue dose, i.e., the average amount metabolized per day per gram of lung (AMPLU) using the PBPK model. The dose metric AMPLU was selected as a surrogate for the target tissues dose based on the mode of action and cancer dose–response analysis (Himmelstein et al., 2004b). The PBPK-derived AMPLU values were calculated using both point- and probability-based metabolic parameters. For the deterministic approach, the updated liver, lung and kidney metabolism parameter estimates from the Nelder-Mead algorithm were scaled allometrically and used in the PBPK model to calculate gender-specific AMPLU values for mice, rats and humans. For the probabilistic approach, the Monte Carlo technique was employed to calculate the distribution of the gender- and species-specific AMPLUs by sampling and scaling from the posterior distributions of the metabolism parameters for liver, lung and kidney estimated during the MCMC analysis of in vitro metabolisms parameters.

3. Results

3.1. Microsomal oxidation of chloroprene in liver, lung and kidney tissues

The purpose of this study was to investigate the species, gender, and tissue differences in chloroprene metabolism. To this end, gender-specific microsomal oxidation in liver, lung and kidney were measured in rat, mice, and human using pooled microsomal samples. Estimates of metabolic rate parameters were based on two-compartment modeling of a family of time course curves for each experimental factor (species, sex, and tissue type). The gender- and species-specific metabolic clearance of chloroprene in microsomes is shown by the data points in Fig. 2–5, which represent the disappearance of chloroprene in the head space of a closed vial system for liver and lung in female rats and mice; kidney microsomes of rodents of both genders, and human kidney microsomes. These data were used to estimate in vitro metabolic parameters for chloroprene as described below.

3.2. Parameter estimation using the deterministic approach

The point estimate of the background loss rate constant was 1.41 L/hr/g. The point estimation results for the microsomal oxidation parameters with background loss rate are presented in Table 3. Oxidation parameter estimates without background loss rate correction were also optimized for comparison purposes. Even with the background loss rate, microsomal oxidation was observed in most of the tissues. In some tissues it was possible to see an impact of considering background loss; for example the estimated intrinsic clearance dropped from 1.3 to 0.9 L/hr/g in the male rat lung microsomal incubations. The greatest impact was for the female mouse kidney where the intrinsic clearance decreased from 0.83 to 0.024 L/hr/g. The comparison of chloroprene headspace measurements and model predictions simulated using point estimates of the model parameters are in the Supplemental data.

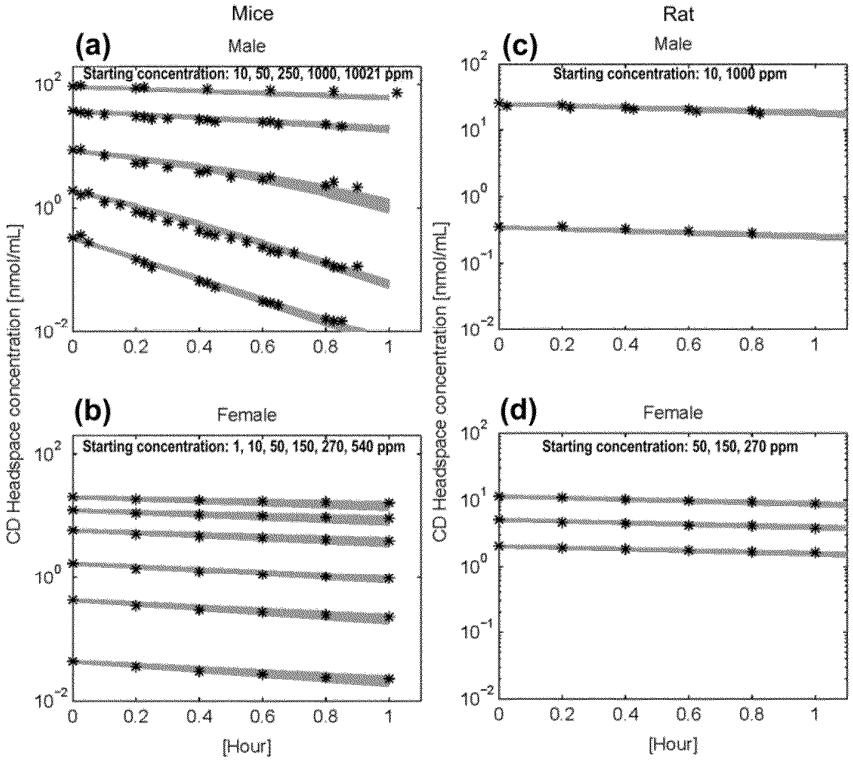

Fig. 3. Distributions of chloroprene oxidative metabolism time-course predictions versus experiment data (symbols) in lung microsomes. Chloroprene headspace concentrations were collected for various starting headspace concentrations. Simulated time course data (lines) were based on posterior distributions for parameter values reported in Table 5. Simulations represent 250 sets of model parameters randomly drawn from the posterior distributions.

3.3. Parameter estimation using the probabilistic approach

Intrinsic clearance (V_{max}/k_m) for background loss was calculated from the geometric mean values for V_{max} and k_m; the resulting 95th, 50th and 5th percentile of the posterior distribution were 1.5, 1.4, and 1.3 L/hr/g, respectively. The convergence of the MCMC results was verified based on CSRF values (see Section 2) which were below 1.1 for all the parameters.

Estimates of the enzyme-mediated metabolic constants are presented in Table 4 The means of the posterior distributions of the metabolic parameters showed excellent agreement with those of the point estimates (Table 3). The point estimates were typically within one standard deviation of the posterior mean values (Table 4). One exception was the intrinsic clearance for the female mouse kidney where the Bayesian estimate (0.25 L/hr/g) was 10-fold higher than the point estimate (0.024 L/hr/g). The uncertainties in the model parameters were significantly reduced from the prior distributions, as demonstrated by the narrower posterior distributions (Table 4). For all species, the metabolic capacity in microsomes was the highest in the liver, followed by the lung or kidney (Tables 3 and 4). Gender differences were observed in all tissues examined (Kruskal–Wallis ANOVA, $p < 0.0001$, Table 4). The intrinsic clearance (V_{max}/k_m) determined in liver microsomes was higher in males than in females both for rats and mice. Species differences in the tissue intrinsic clearance rate were also observed. Higher clearance was estimated in the lung than the kidney for mice; but this was reversed for rats (Tables 3 and 4). Figs. 2–5 present the distributions of the rate of chloroprene metabolism simulated with metabolic constants randomly drawn from their posterior distributions. The width of the band showing 250 randomly selected simulations reflects the impact of the uncertainty of the metabolic parameters on the distribution of the model output, i.e., chloroprene concentration in this case. The point estimation and Bayesian method both provided good agreement with the in vitro experiential observations.

3.4. Internal dose calculations

AMPLU was calculated using the PBPK model with the updated metabolic rate constants from the in vitro studies (Table 2). PBPK model-predicted AMPLUs based on the metabolism parameters scaled from both the deterministic and probabilistic approaches were compared (Table 5). The AMPLU values were comparable between the two methods, the means estimated with the point estimation fall within the probabilistic distribution of AMPLUs from the MCMC analysis. Results from both approaches showed that the total metabolism per gram lung was greatest in mice follow by rats and humans. Both approaches indicated that AMPLU was linear for the rats and human over the selected bioassay concentrations (12.8, 32, or 80 ppm) but indicated saturation for the mouse, consistent with the previous dose–response analysis for male rats and mice (Himmelstein et al., 2004b). A gender difference in the AMPLU estimates was observed for both rats and mice; however, they were more significant in mice (male mouse AMPLU was 4–5 time higher than the values estimated for female mouse).

4. Discussion

One of the major challenges in using PBPK models in risk assessment is the issue of uncertainty and variability in model predictions. Until recently, most of the PBPK models have been developed based on point estimates for the physiological and chemical-specific

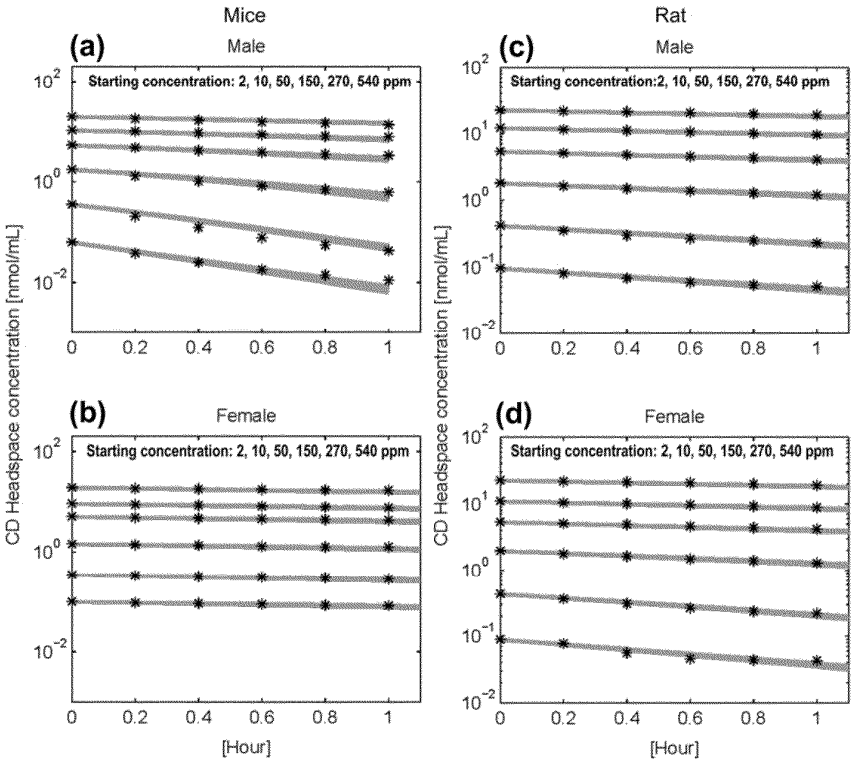

Fig. 4. Distributions of chloroprene oxidative metabolism time-course predictions versus experiment data (symbols) in kidney microsomes. Chloroprene headspace concentrations were collected using various starting headspace concentrations. Simulated time course data (lines) were based on posterior distributions for parameter values reported in Table 5. Simulations represent 250 sets of model parameters randomly drawn from the posterior distributions.

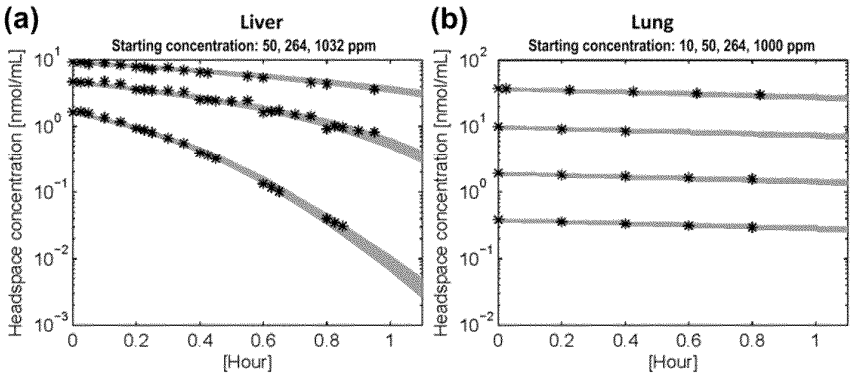

Fig. 5. Distribution of chloroprene oxidative metabolism time course predictions in liver and kidney microsomes for human. Symbols represent measured chloroprene headspace concentrations. Model simulations (lines) were based on posterior distributions of parameter values as reported in Table 5. Simulations represent 250 sets of model parameters randomly drawn from the posterior distributions.

parameter values, and consequently have predicted a single kinetic behavior of the chemical in the body. However, differing degrees of uncertainty are expected both for physiological and chemical-specific parameter estimates, especially metabolism constants, which will result in a corresponding range of model predictions for the dose metric of interest. For the purposes of this investigation – evaluating the risks of chloroprene exposure – the issue to be addressed is the impact of true uncertainty (uncertainty regarding the central estimate of a particular metabolic parameter in a given species, gender, and tissue) on risk estimates (target tissue dosimetry). No attempt was made to characterize population variability in either the rodent or the human. Risk assessments for chloroprene have been based on summarized outcomes (incidence) in groups of animal or workers, and the goal of these assessments has been to provide a central or upper-bound estimate of the risk to an average individual. Therefore, our focus on true uncertainty is appropriate. Although the National Academy of Science (NAS, 2009) has recently recommended greater consideration of human variability in risk assessment, this would require a much more ambitious experimental study involving a large number of human tissues.

In an effort to characterize the impact of metabolic parameter uncertainty on a risk assessment for chloroprene, we applied a

Table 2
In vivo PBPK model parameters.

Parameter	Mouse		Fischer rat		Human
Body weight (kg)	0.03		0.25		70
Ventilation (L/h/kg$^{0.75}$)	30		21		16
Cardiac output (L/h/kg$^{0.75}$)	30		18		16.2
Tissue volumes (%BW)					
Liver	5.5%		4.0%		2.6%
Kidney	1.4%		1.0%		1.0%
Fat	5.0%		7.0%		21.4%
Rapidly perfused	1.4%		3.5%		9.1%
Slowly perfused	77.0%		75.0%		56.1%
Lung	0.7%		0.5%		0.8%
Blood flows (%CO[a])					
Liver	16.1%		18.3%		22.7%
Kidney	10.0%		14.0%		14.0%
Fat	7.0%		7.0%		5.2%
Rapid perfused	51.9%		45.7%		33.2%
Slow perfused	15.0%		15.0%		24.9%
Partition coefficients					
Blood:air	7.83		7.35		4.54
Liver:blood	1.25		1.57		1.44
Kidney:blood	1.76		2.27		2.64
Fat:blood	17.29		16.87		28.38
Rapid perfused:blood	1.76		2.27		2.64
Slow perfused:blood	0.58		0.60		0.99
Lung:blood	2.38		1.84		2.92
Metabolism	Male	Female	Male	Female	Mixed
$V_{max}C$, Lung	0.60 ± 0.03[b]	0.11 ± 0.05			
k_m, Lung	0.20 ± 0.01	0.25 ± 0.13			
KF, Lung			0.15 ± 0.03	0.16 ± 0.02	0.05 ± 0.04
$V_{max}C$, Liver	18.54 ± 0.75	8.88 ± 0.84	9.48 ± 0.25	9.37 ± 0.52	20.4 ± 0.36
k_m, Liver	0.12 ± 0.008	0.08 ± 0.01	0.05 ± 0.003	0.09 ± 0.006	0.04 ± 0.001
$V_{max}C$, Kidney	0.078 ± 0.007	0.03 ± 0.05	0.018 ± 0.002	0.018 ± 0.002	
k_m, Kidney	0.068 ± 0.008	9.59 ± 44	0.067 ± 0.009	0.053 ± 0.007	

[a] CO – cardiac output.
[b] Mean ± SD from Markov Chain Monte Carlo posterior distribution.

probabilistic Bayesian approach to estimate metabolism parameters from in vitro data in which distributions, rather than point estimates, of estimated parameters (posterior distributions) were generated reflecting the uncertainty in the metabolism parameters. To our knowledge this study marks the first time a probabilistic approach has been employed to estimate a distribution for PBPK model parameters from in vitro metabolism studies. We also compared a deterministic approach, nonlinear optimization, with the probabilistic Bayesian approach. For the deterministic approach, the parameters were optimized to provide the maxima likelihoods between the prediction and the data determined in vitro. In the Bayesian approach, relatively non-informative distributions were used as priors (e.g., uniform distributions) so that the posterior distributions of the parameters would be estimated primarily on the likelihood of the parameters given the data. Thus, the metabolic parameters were estimated based on data-likeihood regardless of the methods used for parameter optimization. Our results show that the in vitro metabolism parameters obtained from the two different approaches are consistent: the point estimates for the parameters from the deterministic method are within the posterior distributions obtained from the MCMC analysis. The parallel parameter estimation using both deterministic and the probabilistic methods provided us an opportunity to evaluate the uncertainty in the resulting model parameters while still being able to compare the results from the current modeling to those from the previous modeling effort in which the parameters were optimized by a deterministic approach.

The estimated uncertainty in the in vitro metabolism of chloroprene was higher in female than male rodents. The uncertainty in the data was likely smaller in the males due to the greater amount of data available. In the case of kidney, the same numbers of measurement were collected for both genders. In this case, the larger uncertainty observed with the in vitro-data for female mice may result from the lower rates of chloroprene metabolism in kidney microsomes from female mice. The lower clearance of chloroprene by kidney makes it more difficult to distinguish metabolism from background vial loss. This lower rate of kidney metabolism in the female mouse is not unexpected since chloroprene inhalation caused kidney toxicity in male, but not female, mice (Melnick et al., 1999).

Our probabilistic and deterministic approaches resulted in similar estimates for parameter values. However, the use of a probabilistic approach allowed us to evaluate the uncertainty in the estimates of dose metric, in this case AMPLU, as a result of uncertainty in the in vitro data. To accomplish this, the PBPK model and the posteriors of metabolic PBPK model parameters (liver, lung, and kidney) from the in vitro MCMC analysis were used to simulate the distribution of AMPLU via Monte Carlo techniques. The resulting variance of the AMPLU distribution, represented by the coefficient of variation (CV), increased with exposure concentration in the case of mice; but not for rats and humans. For mice, the CV was doubled when exposure concentration was increased from 12.8 to 80 ppm in both genders (3.8%–7.6% for male and 11%–24% for female). For rats, the CV was about 20% for females for the three concentrations tested, which were slightly higher than male rats (about 14%). This difference likely reflects the fact that lung metabolism in mice was described as a saturable (non-linear) pathway while a linear pathway was used for rat and human. For humans, the AMPLU values were the lowest among the species, and the population variation of AMPLU attributable to the uncertainty in the metabolism

Table 3
Point estimate values for the microsomal oxidation of chloroprene.

Species	Sex	Tissue	Metabolic parameters[a,b]		
			V_{max}	k_m	V_{max}/k_m
B6C3F1 mouse	Male	Liver	0.26	1.36	186
		Lung	0.13	2.0	64
		Kidney	0.01	0.5	20
	Female	Liver	0.09	0.53	174
		Lung	0.025	2.78	8.9
		Kidney	0.00004	1.7	0.024
F344 rat	Male	Liver	0.077	0.56	139
		Lung			0.9
		Kidney	0.0027	0.92	3
	Female	Liver	0.068	0.82	82
		Lung			1.2
		Kidney	0.00177	0.37	4.7
Human	Mixed	Liver	0.054	0.45	120
		Lung			0.9

[a] Obtained by ACSL Optimization and includes correction for background loss of chloroprene during the incubation.
[b] Units: V_{max} (lmol/h/mg); k_m (lmol/L); V_{max}/k_m (L/hr/g).

parameters was most significant (over 60%). The variation of the intrinsic clearance for the human lung was also the highest among tissues and species.

Overall, we used MCMC analysis as a probabilistic parameter optimization tool to provide estimates of metabolic parameter distributions for use in a PBPK model. This approach is consistent with the EPA's efforts to develop guidelines for probabilistic risk assessment. In the recent EPA "Risk Assessment Guidance for Superfund", it states "... methods used to quantify uncertainty in the model inputs are based on statistical principles such as sampling distributions (Monte Carlo analysis) or Bayesian approaches". In a Monte Carlo probability analysis (Buur et al., 2006; David et al., 2006; Thomas et al., 1996), the parameter variability was estimated by 'assigning' distributions around point estimates obtained from literature reviews or in vitro data. Our approach used Bayesian techniques to generate the probabilistic distributions of model parameters based on statistical procedure and reflected the prior knowledge (when available) and the data from new experimental studies. It should be noted, however, that the posteriors from Bayesian analyses are calibrated to a particular data set, consideration must be given as to whether the subject populations in the data sets represent the population(s) of interest. For example, one would expect low variability of the kinetic parameters derived from animal experiments since the laboratory animals are more homogeneous. However, tissues in a human study may be less representative of the general population due to human heterogeneity. If there is interest in accounting for variability in the risk assessment, traditional Monte Carlo simulation can be performed where some of the Bayesian-based posterior distributions are replaced with distributions considered more representative of the population of interest (such as the defined population variability of CYP-mediate metabolism based on CYP polymorphisms and abundance (Lipscomb et al., 2004)).

In summary, this study presents a novel probabilistic approach to integrate in vitro metabolism data with physiologically based pharmacokinetic (PBPK) modeling. To achieve this goal, first, gender-specific in vitro microsomal data were collected in liver, lung and kidney for mice, rats, and humans. Second, gender- and tissue-specific metabolism parameters were estimated using a compartmental pharmacokinetic (PK) model and Bayesian analysis. Central estimation of the posterior distributions of parameters from the Bayesian analysis were compared to parameters obtained using a traditional optimization method to provide confidence in the values obtained. Third, the role of metabolism differences in species- and sex-dependent tissue dose metrics were investigated by running the PBPK model with the posterior parameter distributions. Our results show that in vitro-derived metabolism rate constant distributions can be linked in PBPK models to evaluate the role of metabolism differences in species- and sex-dependent tissue dose metrics, further to evaluate the resulting uncertainty in risk estimates of chloroprene.

Table 4
Probability analysis of microsomal oxidation parameters for chloroprene.

Species	Sex	Tissue	Metabolic constants[a]					
			V_{max}[b]		k_m[b]		V_{max}/k_m[c,d,e]	
			Mean	SD	Mean	SD	Mean	SD[d]
B6C3F1	Male	Liver	0.26	0.01	1.34	0.08	194.7	5.50
Mouse		Lung	0.14	0.01	2.22	0.14	63.7	1.00
		Kidney	0.01	0.001	0.77	0.09	16.8	0.70
	Female	Liver	0.13	0.01	0.88	0.14	144.5	11.80
		Lung	0.03	0.01	2.82	1.51	9.7	1.10
		Kidney	0.004	0.01	176.11	922.87	0.25	0.26
F344	Male	Liver	0.10	0.003	0.56	0.03	138	3.65
Rat		Lung					1.28	0.25
		Kidney	0.003	0.0003	0.76	0.11	3.3	0.18
	Female	Liver	0.09	0.01	0.56	0.03	78.6	1.75
		Lung					0.96	0.22
		Kidney	0.003	0.0003	0.60	0.08	4.2	0.16
Human	Mixed	Liver	0.05	0.001	0.45	0.01	122.2	2.20
		Lung					0.32	0.2
		Kidney						ND[f]

[a] Mean (exp(m)) and standard deviation SD (exp(s)) values obtained by Markov Chain Monte Carlo (MCMC) analysis and includes correction for background loss of chloroprene during the incubation.
[b] V_{max} (lmol/h/mg); k_m (lmol/L).
[c] V_{max}/k_m (L/hr/g) calculated as V_{max}/k_m*1000 mg/g (unit conversion).
[d] Mean and SD V_{max}/k_m estimated directly via MCMC analysis.
[e] ND – metabolism not detected.
[f] Tissue-specific microsomal activities were significantly different between the gender for rat and mice (Kruskal–Wallis ANOVA (nonparametric), $p < 0.0001$) (Supplemental data C).

Table 5
Estimation of PBPK-derived internal dose in the lung using deterministic and probabilistic approaches.

Species	Exposure conc. (ppm)	Gender	Internal dose[a]					
			Deterministic Approach[b]	Probabilistic approach[c]				
				Mean	CV%	5%	50%	95%
B6C3F1	12.8	Male	4.15	4.17	2.2	4.02	4.17	4.31
Mouse		Female	0.74	0.79	10.9	0.65	0.79	0.92
	32	Male	6.66	6.86	3.3	6.57	6.94	7.26
		Female	1.19	1.29	16.5	0.99	1.25	1.67
	80	Male	8.56	8.99	4.4	8.49	9.07	9.76
		Female	1.58	1.53	24.1	1.19	1.64	2.51
F344	12.8	Male	0.19	0.24	17.5	0.17	0.24	0.31
Rat		Female	0.23	0.26	14.2	0.20	0.26	0.33
	32	Male	0.47	0.60	18.7	0.41	0.60	0.77
		Female	0.56	0.67	14.3	0.51	0.67	0.83
	80	Male	1.18	1.54	18.4	1.02	1.57	1.96
		Female	1.42	1.69	14.5	1.29	1.68	2.11
Human	12.8	Mixed	0.1	0.04	64.5	0.01	0.04	0.11
	32	Mixed	0.25	0.09	67.1	0.02	0.08	0.28
	80	Mixed	0.64	0.23	64.0	0.04	0.20	0.65

[a] Internal dose = average daily umole CD metabolized/g lung tissue.
[b] Calculated based on point-estimates of parameters (Table 3).
[c] Calculated based on posterior distributions of tissue-specific metabolism parameters (Table 4).

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgements

The authors would like to thank Drs. Miyoung Yoon, Rebecca Clewell and Mel Andersen from the Hamner Institutes for Health Sciences for the detailed review of the draft manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tiv.2012.04.004.

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To: Shelow, David[Shelow.David@epa.gov]
From: Weinstock, Lewis
Sent: Wed 12/9/2015 2:01:40 PM
Subject: RE: Wind rose

Have never seen a rose with such a small westerly component.

Lewis Weinstock | Group Leader | Ambient Air Monitoring Group | Air Quality Assessment Division - Mail Code C304-06 | Office of Air Quality Planning & Standards | U.S. Environmental Protection Agency | Research Triangle Park, NC 27711 | Phone: 919-541-3661|

From: Shelow, David
Sent: Wednesday, December 09, 2015 7:59 AM
To: Palma, Ted <Palma.Ted@epa.gov>; Strum, Madeleine <Strum.Madeleine@epa.gov>; Smith, Darcie <Smith.Darcie@epa.gov>; Rimer, Kelly <Rimer.Kelly@epa.gov>
Cc: Weinstock, Lewis <Weinstock.Lewis@epa.gov>; Scheffe, Rich <Scheffe.Rich@epa.gov>; Wayland, Richard <Wayland.Richard@epa.gov>; Merrill, Raymond <Merrill.Raymond@epa.gov>
Subject: FW: Wind rose

I asked Mark Evangelista to create wind roses, annual and seasonal, for the chloroprene DuPont site in LaPlace LA. This could be helpful if there is monitoring to be done at the elementary school.

Dave

David M. Shelow
National Air Toxics Ambient Monitoring Program Manager
U.S. Environmental Protection Agency
Office of Air Quality Planning and Standards
Ambient Air Monitoring Group C304-06
Research Triangle Park, NC 27711
Phone: 919-541-3776
Fax:: 919-541-1903
Email: shelow.david@epa.gov

From: Evangelista, Mark
Sent: Tuesday, December 08, 2015 4:13 PM
To: Shelow, David <Shelow.David@epa.gov>
Subject: Wind rose

To: France, Danny[France.Danny@epa.gov]
From: Shelow, David
Sent: Wed 12/16/2015 4:07:00 PM
Subject: RE: West Louisville report

One more question… this report and data corresponds to work done in CY2000 – 2001. We have some summary report from data in CY2004. Did Region 4 do work in Rubbertown near the DuPont facility in 2004? If not who did? Kentucky?

Thanks,

Dave

David M. Shelow
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Phone: 919-541-3776
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Email: shelow.david@epa.gov

From: France, Danny
Sent: Wednesday, December 16, 2015 8:49 AM
To: Shelow, David <Shelow.David@epa.gov>; Noah, Greg <Noah.Greg@epa.gov>
Subject: RE: West Louisville report

From: Shelow, David
Sent: Wednesday, December 16, 2015 7:04 AM
To: France, Danny <France.Danny@epa.gov>; Noah, Greg <Noah.Greg@epa.gov>
Subject: RE: West Louisville report

Hi Danny,

Yes we would need the raw data or the appendices to see the actual data. If you have the data I would love to see it.

Dave

David M. Shelow
National Air Toxics Ambient Monitoring Program Manager
U.S. Environmental Protection Agency
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Research Triangle Park, NC 27711
Phone: 919-541-3776
Fax:: 919-541-1903
Email: shelow.david@epa.gov

From: France, Danny
Sent: Tuesday, December 15, 2015 3:59 PM
To: Noah, Greg <Noah.Greg@epa.gov>; Shelow, David <Shelow.David@epa.gov>
Subject: RE: West Louisville report

As the report says, we did see 2-CHLORO-1,3-BUTADIENE (CHLOROPRENE).

Let me know if you need raw data.

From: Noah, Greg
Sent: Tuesday, December 15, 2015 3:47 PM
To: France, Danny <France.Danny@epa.gov>; Shelow, David <Shelow.David@epa.gov>
Subject: RE: West Louisville report

Thanks! I hope it does.

From: France, Danny
Sent: Tuesday, December 15, 2015 3:46 PM
To: Noah, Greg <Noah.Greg@epa.gov>; Shelow, David <Shelow.David@epa.gov>
Subject: West Louisville report

Got your message. Hope this helps.

Danny

Danny France

Chief, Analytical Services Branch

Region 4, EPA, Science and Ecosystems Support Division

980 College Station Rd

Athens, GA 30605

(706) 355-8738 (office)

To: Smith, Darcie[Smith.Darcie@epa.gov]; Strum, Madeleine[Strum.Madeleine@epa.gov]; Palma, Ted[Palma.Ted@epa.gov]; Rimer, Kelly[Rimer.Kelly@epa.gov]; Merrill, Raymond[Merrill.Raymond@epa.gov]; Lassiter, Penny[Lassiter.Penny@epa.gov]; Bremer, Kristen[Bremer.Kristen@epa.gov]
Cc: Wayland, Richard[Wayland.Richard@epa.gov]; Weinstock, Lewis[Weinstock.Lewis@epa.gov]
From: Shelow, David
Sent: Tue 12/15/2015 8:49:46 PM
Subject: FW: West Louisville report

Here is the report from Region 4 for Louisville Kentucky chloroprene study at DuPont in KY.

David M. Shelow
National Air Toxics Ambient Monitoring Program Manager
U.S. Environmental Protection Agency
Office of Air Quality Planning and Standards
Ambient Air Monitoring Group C304-06
Research Triangle Park, NC 27711
Phone: 919-541-3776
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Email: shelow.david@epa.gov

From: France, Danny
Sent: Tuesday, December 15, 2015 3:46 PM
To: Noah, Greg <Noah.Greg@epa.gov>; Shelow, David <Shelow.David@epa.gov>
Subject: West Louisville report

Got your message. Hope this helps.

Danny

Danny France

Chief, Analytical Services Branch

Region 4, EPA, Science and Ecosystems Support Division

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Athens, GA 30605

(706) 355-8738 (office)

To: Palma, Ted[Palma.Ted@epa.gov]; Strum, Madeleine[Strum.Madeleine@epa.gov]; Smith, Darcie[Smith.Darcie@epa.gov]; Rimer, Kelly[Rimer.Kelly@epa.gov]
Cc: Weinstock, Lewis[Weinstock.Lewis@epa.gov]; Scheffe, Rich[Scheffe.Rich@epa.gov]; Wayland, Richard[Wayland.Richard@epa.gov]; Merrill, Raymond[Merrill.Raymond@epa.gov]
From: Shelow, David
Sent: Wed 12/9/2015 12:59:08 PM
Subject: FW: Wind rose

I asked Mark Evangelista to create wind roses, annual and seasonal, for the chloroprene DuPont site in LaPlace LA. This could be helpful if there is monitoring to be done at the elementary school.

Dave

David M. Shelow
National Air Toxics Ambient Monitoring Program Manager
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Phone: 919-541-3776
Fax:: 919-541-1903
Email: shelow.david@epa.gov

From: Evangelista, Mark
Sent: Tuesday, December 08, 2015 4:13 PM
To: Shelow, David <Shelow.David@epa.gov>
Subject: Wind rose

To: Bremer, Kristen[Bremer.Kristen@epa.gov]; Millett, John[Millett.John@epa.gov]
Cc: Smith, Darcie[Smith.Darcie@epa.gov]; Rimer, Kelly[Rimer.Kelly@epa.gov]; Noonan, Jenny[Noonan.Jenny@epa.gov]
From: Gray, David
Sent: Mon 12/7/2015 7:21:33 PM
Subject: RE: Draft Communications Plan for LaPlace, LA

FYI for the 114 letter. The sources at this facility are broader than stacks. Attached is a break out from our folks.

Attached is the 2014 LDEQ emissions inventory for chloroprene sources at the Dupont facility in LaPlace, LA. In summary about 24 tons are being emitted from area/fugitive sources such as building exhaust fans, 21 tons are being released from vents, and 84 tons are coming from stacks. Approximately half the emissions are coming from the top 4 to 5 sources, however in order to bring risk levels down to our typically evaluated regulatory levels of concern (1 to 100 in a million), the top 40 sources need to be evaluated.

To: Stenger, Wren[stenger.wren@epa.gov]
From: Casso, Ruben
Sent: Tue 12/15/2015 10:29:08 PM
Subject: RE: DuPont/Denka
contact info.docx

Not sure what 6EN is calling themselves since the re-org. Filled in the rest.

From: Stenger, Wren
Sent: Tuesday, December 15, 2015 4:06 PM
To: Casso, Ruben
Cc: Verhalen, Frances
Subject: DuPont/Denka

Ruben, please check my inputs and complete this table to include all program contacts needed for DuPont/Denka activities. Thanks

To: Casso, Ruben[Casso.Ruben@epa.gov]
From: Smith, Darcie
Sent: Thur 11/19/2015 6:32:47 PM
Subject: update

Hi Ruben –

Ted Palma had a call from Jeff Yurk (R6, enforcement) to ask about the chloroprene facility. We have not been able to get in touch with David Gray or Wren Stingler (sp??), but have calls into both of them. If you like, we can get all the Region 6 folks together and give you all an update at the same time. It would be the same information you currently have, so maybe you would like to do that, but if you want us to pull something together, please let me know. Just an offer.

Fyi - We sent some materials – basic NATA modeling steps/instructions and a kmz of ambient chloroprene concentrations – to DuPont representatives today, as a follow up to our call with them on Tuesday.

Darcie

Darcie Smith

U.S. EPA/OAQPS/HEID/ATAG

Mail Drop C539-02

109 TW Alexander Dr.

RTP, NC 27711

(919) 541-2076

To: Ted Palma[Palma.Ted@epa.gov]
From: Smith, Darcie
Sent: Thur 7/30/2015 3:46:20 PM
Subject: NATA facility

Hi Ted –

Today is Steve's birthday.

He said you were having trouble with a facility emitting chloroprene, in LA. I think it is a Polymers and Resins I – Neoprene facility. We modeled it in P&R I RTR, but there was not a chloroprene URE at the time so it didn't show up with high cancer risk, although it did have lots of emissions. Anyway, just an FYI.

Darcie

Darcie Smith

U.S. EPA/OAQPS/HEID/ATAG

Mail Drop C539-02

109 TW Alexander Dr.

RTP, NC 27711

(919) 541-2076

To: Chance McNeely - LDEQ assistant secretary[deqoec@la.gov]; Cheryl Nolan[tegan.treadaway@la.gov]
From: Stenger, Wren
Sent: Thur 12/17/2015 7:38:38 PM
Subject: Chloroprene

Just heard from David Gray that the NATA will be released at 3 PM eastern (2 pm our time, about 30 minutes from now.)

Wren Stenger

Director

Multimedia Planning and Permitting Division

EPA Region 6 Dallas, Texas

214.665.6583

To: Hansen, Mark[Hansen.Mark@epa.gov]; Verhalen, Frances[verhalen.frances@epa.gov]; Casso, Ruben[Casso.Ruben@epa.gov]; Thompson, Steve[thompson.steve@epa.gov]; Blevins, John[Blevins.John@epa.gov]; Arturo Blanco (Blanco.Arturo@epa.gov)[Blanco.Arturo@epa.gov]; Anderson, Israel[Anderson.Israel@epa.gov]; Runnels, Charlotte[Runnels.Charlotte@epa.gov]; Gray, David[gray.david@epa.gov]
Cc: Stenger, Wren[stenger.wren@epa.gov]
From: Stenger, Wren
Sent: Thur 12/17/2015 5:28:50 PM
Subject: DuPont/Denka contacts for Pontchartrain Works

All, here are the contacts all in one place to post, share, or save.

DuPont

Catherine Barton, Catherine.A.Barton@dupont.com, 302.996.8354, issues management.

Lori Sanders, Lori.E.Sanders@dupont.com, 302.996.8276, legal matters.

Tara Stewart, Tara.C.Stewart@dupont.com, 302.358.4012, public affairs.

Denka

Jorge Lavastida, Jorge-Lavastida@denka-pe.com, 225.773.0545, Plant Manager.

Wren Stenger

Director

Multimedia Planning and Permitting Division

EPA Region 6 Dallas, Texas

214.665.6583

To: Catherine.A.Barton@dupont.com[Catherine.A.Barton@dupont.com]; Jorge Lavastida[Jorge-Lavastida@denka-pe.com]
Cc: Blevins, John[Blevins.John@epa.gov]; Arturo Blanco (Blanco.Arturo@epa.gov)[Blanco.Arturo@epa.gov]; Stenger, Wren[stenger.wren@epa.gov]; Gray, David[gray.david@epa.gov]
From: Stenger, Wren
Sent: Thur 12/17/2015 5:23:13 PM
Subject: R6 Contracts for Dupont/Denka Chloroprene/Pontchartrain Works
NATA Chloroprene R6 contacts Dec 17 2015.docx

Catherine, Jorge,

Thanks for your contact information. Here are the R6 contacts. I am also copying the R6 directors as FYI. We look forward to working with you on the chloroprene data and your facility operations.

Wren Stenger

Director

Multimedia Planning and Permitting Division

EPA Region 6 Dallas, Texas

214.665.6583

To: Casso, Ruben[Casso.Ruben@epa.gov]
From: Stenger, Wren
Sent: Mon 12/14/2015 7:49:38 PM

What is the new IRIS number for chloroprene?

Sent from my Windows Phone

To: Blanco, Arturo[Blanco.Arturo@epa.gov]
From: Stenger, Wren
Sent: Fri 12/11/2015 10:18:59 PM
Subject: RE: Chloroprene DuPont NATA LDEQ/LDHH brief

Thanks

Wren Stenger

Director

Multimedia Planning and Permitting Division

EPA Region 6 Dallas, Texas

214.665.6583

From: Blanco, Arturo
Sent: Friday, December 11, 2015 2:17 PM
To: Stenger, Wren
Subject: RE: Chloroprene DuPont NATA LDEQ/LDHH brief

Wren,

Charlotte Runnels and I will join the call. Israel and Rhonda will be out of office.

Arturo

Arturo J. Blanco
Director

Office of Environmental Justice, Tribal and International Affairs

US EPA Region 6

1445 Ross Avenue (6RA-DA)

Dallas, TX 75202

214.665.3182 (o)

Ex. 6 - Personal Privacy (m)

From: Stenger, Wren
Sent: Friday, December 11, 2015 12:31 PM
To: Gray, David; Coleman, Sam
Cc: Hansen, Mark; Williams, Odessa; Blanco, Arturo; Blevins, John; Seager, Cheryl; Edlund, Carl; Garcia, David; Gilrein, Stephen; Harrison, Ben; Hill, Troy; Honker, William; McDonald, James; Phillips, Pam; Smith, Rhonda; Taheri, Diane
Subject: Chloroprene DuPont NATA LDEQ/LDHH brief

David, for the call with LDEQ and LDHH on Monday, Dec 14 at 10 AM, my suggestion for those to be included on the invitation include:

Mike Koerber

Steve Page

Peter Tsirigotis

Penny Lassiter

Kelly Rhimer

Erika Sasser

Others from HQs? Millet, Jenny Noonan, Debbie Jordan, others???

George Pettigrew, Jennifer Lyke

Others from ATSDR or CDC?

Ron Curry, Sam Coleman, David Gray

Wren Stenger, Mark Hansen, Fran Verhalen, Ruben Casso

John Blevins, Steve Gilrein, Steve Thompson, Jeff Yurk

James McDonald, Troy Hill, Wes McQuiddy, Marvelyn Humphrey

Carl Edlund, Ronnie Crossland, Nick Fressia

Ben Harrison, Cheryl Seager

Arturo Blanco, Rhonda Smith, Israel Anderson

Others from R6?

LDEQ and LDHH will provide their names to you directly.

Wren Stenger

Director

Multimedia Planning and Permitting Division

EPA Region 6 Dallas, Texas

214.665.6583

From: Gray, David
Sent: Friday, December 11, 2015 11:56 AM

To: Tegan Treadaway; Stenger, Wren; Coleman, Sam; Noonan, Jenny
Subject: Re: NATA LDEQ/LDHH brief

We have the briefing set up for Monday at 10 am CT. We will need a list of attendees in advance of the meeting so they can access the webinar presentation.

Please send names to me and Jenny Noonan.

Below are details.

For this meeting with the Departments of Environment and Health for the State of Louisiana, we will be using the call in number Ex. 6 - Personal Privacy

To view the webinar: Ex. 6 - Personal Privacy

This has been set up such that only "approved guests" can enter; everyone will need to sign in and be approved by the OAPQS moderators (Kelly and me) before they can enter the meeting. We can approve in the moments before the meeting starts. Everyone should sign in with his/her full names so that we don't have to guess who's trying to enter.

Sent from my iPhone

On Dec 10, 2015, at 4:16 PM, Tegan Treadaway <Tegan.Treadaway@LA.GOV> wrote:

> If not/ please let us know what works. DHH is not available in the pm.
>
> Sent from my iPhone

To: Gray, David[gray.david@epa.gov]; Coleman, Sam[Coleman.Sam@epa.gov]; Blevins, John[Blevins.John@epa.gov]; Edlund, Carl[edlund.carl@epa.gov]; McDonald, James[McDonald.James@epa.gov]; Curry, Ron[Curry.Ron@epa.gov]; Arturo Blanco (Blanco.Arturo@epa.gov)[Blanco.Arturo@epa.gov]; Tsirigotis, Peter[Tsirigotis.Peter@epa.gov]; Page, Steve[Page.Steve@epa.gov]; Koerber, Mike[Koerber.Mike@epa.gov]
From: Stenger, Wren
Sent: Fri 12/11/2015 9:42:22 PM
Subject: Chloroprene DuPont Denka

Dow and DuPont completed negotiations for a merger as of today, according to the business news channels.

Wren Stenger

Director

Multimedia Planning and Permitting Division

EPA Region 6 Dallas, Texas

214.665.6583

To: Gray, David[gray.david@epa.gov]; Coleman, Sam[Coleman.Sam@epa.gov]
Cc: Hansen, Mark[Hansen.Mark@epa.gov]; Williams, Odessa[Williams.Odessa@epa.gov]; Arturo Blanco (Blanco.Arturo@epa.gov)[Blanco.Arturo@epa.gov]; Blevins, John[Blevins.John@epa.gov]; Cheryl Seager (seager.cheryl@epa.gov)[seager.cheryl@epa.gov]; Edlund, Carl[edlund.carl@epa.gov]; Garcia, David[Garcia.David@epa.gov]; Gilrein, Stephen[gilrein.stephen@epa.gov]; Harrison, Ben[Harrison.Ben@epa.gov]; Hill, Troy[Hill.Troy@epa.gov]; Honker, William[honker.william@epa.gov]; McDonald, James[McDonald.James@epa.gov]; Phillips, Pam[phillips.pam@epa.gov]; Rhonda Smith[Smith.rhonda@epa.gov]; Taheri, Diane[Taheri.Diane@epa.gov]
From: Stenger, Wren
Sent: Fri 12/11/2015 6:31:29 PM
Subject: Chloroprene DuPont NATA LDEQ/LDHH brief

David, for the call with LDEQ and LDHH on Monday, Dec 14 at 10 AM, my suggestion for those to be included on the invitation include:

Mike Koerber

Steve Page

Peter Tsirigotis

Penny Lassiter

Kelly Rhimer

Erika Sasser

Others from HQs? Millet, Jenny Noonan, Debbie Jordan, others???

George Pettigrew, Jennifer Lyke

Others from ATSDR or CDC?

Ron Curry, Sam Coleman, David Gray

Wren Stenger, Mark Hansen, Fran Verhalen, Ruben Casso

John Blevins, Steve Gilrein, Steve Thompson, Jeff Yurk

James McDonald, Troy Hill, Wes McQuiddy, Marvelyn Humphrey

Carl Edlund, Ronnie Crossland, Nick Fressia

Ben Harrison, Cheryl Seager

Arturo Blanco, Rhonda Smith, Israel Anderson

Others from R6?

LDEQ and LDHH will provide their names to you directly.

Wren Stenger

Director

Multimedia Planning and Permitting Division

EPA Region 6 Dallas, Texas

214.665.6583

From: Gray, David
Sent: Friday, December 11, 2015 11:56 AM
To: Tegan Treadaway; Stenger, Wren; Coleman, Sam; Noonan, Jenny
Subject: Re: NATA LDEQ/LDHH brief

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To view the webinar, Ex. 6 - Personal Privacy

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Sent from my iPhone

On Dec 10, 2015, at 4:16 PM, Tegan Treadaway <Tegan.Treadaway@LA.GOV> wrote:

> If not/ please let us know what works. DHH is not available in the pm.
>
> Sent from my iPhone

To: Strum, Madeleine[Strum.Madeleine@epa.gov]
From: Thurman, James
Sent: Tue 9/8/2015 4:50:01 PM
Subject: DuPont fan sources
dupont_chloroprene.xlsx

Here are the 17 fans that went to the source with agency release point id of 'PR0185', 2nd row of the file "EPA Modeling SpreadsheetDuPont.xlsx". They total to the 15.83 tons. I didn't see the fans in the pink rows but maybe I don't know what to look for. The lat/lon coordinates in the attached spreadsheet are those based on what Dupont sent. I modeled them as volume sources and the appropriate source characteristics (emissions in g/s , height, sigma-y, and sigma-z) are highlighted in yellow (last 4 columns).

Let me know if questions.

James A. Thurman, Ph.D.

U.S. EPA/OAQPS/AQAD

Air Quality Modeling Group (C439-01)

109 T.W. Alexander Drive

Research Triangle Park, NC 27711

Phone: (919) 541-2703

Fax: (919) 541-0044

Email: thurman.james@epa.gov

To: Strum, Madeleine[Strum.Madeleine@epa.gov]
From: Doris.B.Grego@dupont.com
Sent: Wed 7/15/2015 4:28:40 PM
Subject: Chloroprene Concentration

Madeleine, what is the chloroprene concentration being used to determine the risk?

Thanks,

Doris B. Grego, P.E.

Senior Environmental Consultant

985-536-5437

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To: Strum, Madeleine[Strum.Madeleine@epa.gov]; Thurman, James[Thurman.James@epa.gov]; Kelly.Petersen@LA.gov[Kelly.Petersen@LA.gov]
From: Doris.B.Grego@dupont.com
Sent: Tue 7/14/2015 2:44:23 PM
Subject: Chloroprene Emissions - DuPont
Fan Drawing.pdf
Poly Building Fans.pdf
Release Point Diagram.pdf
ATT00001.txt

I have converted the attachments I sent previously (Release Point Diagram and Fans location) to pdfs and are attached. I'm also including a Fan Drawing showing three of the Poly Building walls. The fans on the west side are the intake fans, the ones on the east and south walls are the discharge ones. This drawing does not show the fans as they are today, but it might help visualize the building and its venting system.

Doris B. Grego, P.E.
Senior Environmental Consultant
985-536-5437

To: Kelly.Petersen@LA.gov[Kelly.Petersen@LA.gov]
Cc: Strum, Madeleine[Strum.Madeleine@epa.gov]; Thurman, James[Thurman.James@epa.gov]
From: Doris.B.Grego@dupont.com
Sent: Mon 7/13/2015 3:35:28 PM
Subject: RE: DuPont Stack Parameters-- AERMOD modeling of high risk high emitting chloroprene source for NATA

Kelly, I'm available on Wednesday July 15 from 9:00 am to 11:00 am central time (10 to 12 eastern)

Doris B. Grego, P.E.

Senior Environmental Consultant

985-536-5437

From: Kelly Petersen [mailto:Kelly.Petersen@LA.GOV]
Sent: Monday, July 13, 2015 7:51 AM
To: GREGO, DORIS B
Cc: 'Strum, Madeleine'; 'thurman.james@epa.gov'
Subject: FW: DuPont Stack Parameters-- AERMOD modeling of high risk high emitting chloroprene source for NATA

Doris,

EPA has requested a call to be sure they are interpreting your spreadsheet correctly. Are any of the times below convenient for you?

Thanks,

Kelly Petersen

Air Permits Division

Louisiana Department of Environmental Quality

Phone: (225) 219-3397 Fax: (225) 325-8141 kelly.petersen@la.gov

From: Strum, Madeleine [mailto:Strum.Madeleine@epa.gov]
Sent: Monday, July 13, 2015 7:45 AM
To: Kelly Petersen
Cc: Thurman, James
Subject: RE: DuPont Stack Parameters-- AERMOD modeling of high risk high emitting chloroprene source for NATA

Thanks Kelly!

James and I are free:

Wednesday between 10am and 12pm, EDT

Thursday between 1pm and 4pm, EDT

I think an hour will be more than enough.

Madeleine

From: Kelly Petersen [mailto:Kelly.Petersen@LA.GOV]
Sent: Monday, July 13, 2015 8:36 AM
To: Strum, Madeleine
Subject: RE: DuPont Stack Parameters-- AERMOD modeling of high risk high emitting chloroprene source for NATA

I would prefer to just facilitate a call. Can you give me some times that will work for you?

Kelly Petersen

Air Permits Division

Louisiana Department of Environmental Quality

Phone: (225) 219-3397 Fax: (225) 325-8141 kelly.petersen@la.gov

From: Strum, Madeleine [mailto:Strum.Madeleine@epa.gov]
Sent: Monday, July 13, 2015 7:34 AM
To: Kelly Petersen
Subject: FW: DuPont Stack Parameters-- AERMOD modeling of high risk high emitting chloroprene source for NATA

Kelly,

Can you help facilitate a call with you and the facility or do you think you can interpret the information the facility sent and we'd just have the call with you?

Madeleine

Madeleine Strum
U.S. Environmental Protection Agency
Office of Air Quality Planning and Standards/Air Quality Assessment Division/EIAG
919 541 2383 (voice)
919 541 0684 (fax

From: Thurman, James
Sent: Monday, July 13, 2015 8:30 AM
To: Strum, Madeleine
Subject: RE: DuPont Stack Parameters-- AERMOD modeling of high risk high emitting chloroprene source for NATA

We should probably have a quick call with the state/facility to make sure I'm interpreting the spreadsheet correctly.

James A. Thurman, Ph.D.

U.S. EPA/OAQPS/AQAD

Air Quality Modeling Group (C439-01)

109 T.W. Alexander Drive

Research Triangle Park, NC 27711

Phone: (919) 541-2703

Fax: (919) 541-0044

Email: thurman.james@epa.gov

From: Kelly Petersen [mailto:Kelly.Petersen@LA.GOV]
Sent: Tuesday, July 07, 2015 11:22 AM
To: Strum, Madeleine
Subject: FW: DuPont Stack Parameters

From: Doris.B.Grego@dupont.com [Doris.B.Grego@dupont.com]
Sent: Monday, July 06, 2015 1:14 PM
To: Kelly Petersen
Subject: DuPont Stack Parameters

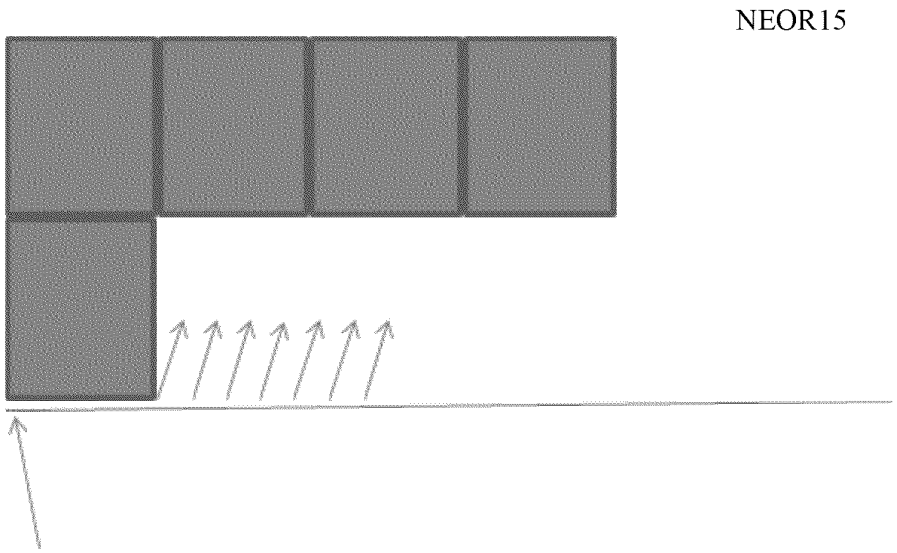
Attached is the revised EPA Modeling spreadsheet for the chloroprene sources at the DuPont Facility located in LaPlace, Louisiana. The changes are in red.

Two items need to be clarified.

1. On the chloroprene tab of the Modeling spreadsheet, the sources highlighted in pink do not discharge directly to the atmosphere, these sources are routed through on the of the vents listed in rows 1 through 39.

For example sources NEO 222 thru 226 (rows 99 to 103) discharge through vent RPN015 which is source NEOR15 (row 1). Only the sources on rows 1 through 39 should be modeled.

See example below.



NEO222 NEO223 NEO224 NEO225 NEO22

2. The second source on the spreadsheet, NEO185, consists of seventeen wall fans located on the Poly Building. Twelve fans are located on the east wall of the building, five are located on the south wall of the building. Attached is an Xcel file which includes two diagrams, one for each wall, and a table with the dimensions, emissions and locations of the fans. The fans are either 8' x 8' or 4' x 4', they are used to pull air from the building to minimize the concentration of chloroprene. For permitting and reporting purposes, I grouped all the fans into one fugitive emission source. For modeling purpose, they should be considered individually.

If you have any questions or need additional information, please let me know.

Doris B. Grego, P.E.

Senior Environmental Consultant

985-536-5437

To: 'doris.b.grego@dupont.com'[doris.b.grego@dupont.com]
Cc: Strum, Madeleine[Strum.Madeleine@epa.gov]; Thurman, James[Thurman.James@epa.gov]
From: Kelly Petersen
Sent: Mon 7/13/2015 12:50:55 PM
Subject: FW: DuPont Stack Parameters-- AERMOD modeling of high risk high emitting chloroprene source for NATA

Doris,

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Thanks,

Kelly Petersen

Air Permits Division

Louisiana Department of Environmental Quality

Phone: (225) 219-3397 Fax: (225) 325-8141 kelly.petersen@la.gov

From: Strum, Madeleine [mailto:Strum.Madeleine@epa.gov]
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Kelly Petersen

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Madeleine

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Office of Air Quality Planning and Standards/Air Quality Assessment Division/EIAG
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James A. Thurman, Ph.D.

U.S. EPA/OAQPS/AQAD

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Email: thurman.james@epa.gov

From: Kelly Petersen [mailto:Kelly.Petersen@LA.GOV]
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To: Strum, Madeleine

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From: Doris.B.Grego@dupont.com [Doris.B.Grego@dupont.com]
Sent: Monday, July 06, 2015 1:14 PM
To: Kelly Petersen
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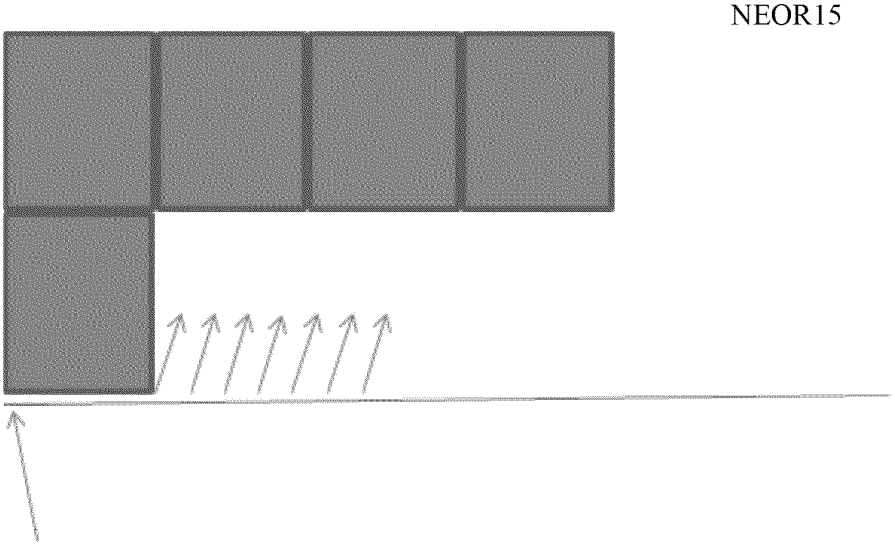

RPN015 is the release point

NEO222 NEO223 NEO224 NEO225 NEO22

2. The second source on the spreadsheet, NEO185, consists of seventeen wall fans located on the Poly Building. Twelve fans are located on the east wall of the building, five are located on the south wall of the building. Attached is an Xcel file which includes two diagrams, one for each wall, and a table with the dimensions, emissions and locations of the fans. The fans are either 8' x 8' or 4' x 4', they are used to pull air from the building to minimize the concentration of chloroprene. For permitting and reporting purposes, I grouped all the fans into one fugitive emission source. For modeling purpose, they should be considered individually.

If you have any questions or need additional information, please let me know.

Doris B. Grego, P.E.

Senior Environmental Consultant

985-536-5437


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Comparison of standardized mortality ratios (SMRs) obtained from use of reference populations based on a company-wide registry cohort to SMRs calculated against local and national rates

Robin C. Leonard [a,*], Kim H. Kreckmann [a], George A. Lineker [b], Gary Marsh [c], Jeanine Buchanich [c], Ada Youk [c]

[a] *DuPont Haskell Laboratory for Health and Environmental Sciences, P.O. Box 50, Newark, DE 19714, United States*
[b] *Peccavis Consulting, Providence, RI 02906, United States*
[c] *University of Pittsburgh, Pittsburgh, PA, United States*

Available online 10 September 2006

Abstract

The DuPont Company has maintained a mortality registry for all active and pensioned U.S. employees since 1957. Standardized mortality ratios (SMRs) for each plant site in the U.S. can be calculated based on the comparison with the entire U.S. DuPont population or with a regional subset of DuPont employees. We compared the SMRs derived from a large, international cohort mortality study of chloroprene workers (IISRP study) with those derived from the entire DuPont Registry and appropriate subpopulations of the registry for two U.S. neoprene plants—Louisville (Kentucky) and Pontchartrain (Louisiana).

SMRs from the IISRP study for the Louisville cohort based on national rates for all causes of death, all cancers, respiratory cancer, and liver cancer are higher than those based on local mortality rates. Both the national and local comparisons (several counties surrounding each plant) for all-cancer SMRs are lower than 1.0, the local comparison being statistically significantly reduced. In contrast, the SMRs based on the total U.S. DuPont worker mortality rates for all causes of death (1.13), all cancers (1.11), and respiratory cancers (1.37) are statistically significantly increased. The SMR for liver cancer (1.27), although elevated, is not statistically significant. SMRs based on DuPont Region 1 were closer to 1.0, and the SMR for all cancers was no longer significant.

Stratification of the Louisville subcohort of males using the same cumulative exposure categories used in the IISRP study yielded SMRs calculated against DuPont Region 1 that were generally higher than those calculated against U.S. and local rates. Only the third exposure category showed SMRs statistically significantly above 1.0 for all cancers and for cancer of bronchus, trachea, and lung. However, there does not appear to be an exposure–response trend.

The SMRs from the IISRP study for the Pontchartrain cohort based on national rates are higher than those based on local rates for all causes of death, but all are less than 1.0. The all-cause SMRs for both local and national comparisons are significantly reduced. There were no deaths from liver cancers observed in this cohort. Comparisons of the Pontchartrain cohort against the total U.S. DuPont worker mortality rates resulted in higher SMRs for all causes of death (0.98), all cancers (1.03), and respiratory cancer (1.08), but none were statistically significant. SMRs based on DuPont Region 2 showed very little change from those based on the total registry.

The use of reference rates based on regional workers in the same large company produces SMRs lower than those based on the entire company population (regional socio-cultural effects) but higher than those based on geographically closer local general populations (healthy worker effect). The healthy worker effect is seen in cancer mortality rates as well as in other chronic diseases.

Keywords: Chloroprene; Healthy worker effect; Standardized mortality ratio; Occupational epidemiology

* Corresponding author. Tel.: +13023666594; fax: +13023665207.
E-mail address: robin.c.leonard@usa.dupont.com (R.C. Leonard).

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doi:10.1016/j.cbi.2006.09.001

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Chemico-Biological Interactions 166 (2007) 285–300
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www.elsevier.com/locate/chembioint

Mortality patterns among industrial workers exposed to chloroprene and other substances I. General mortality patterns

Gary M. Marsh[a,*], Ada O. Youk[a], Jeanine M. Buchanich[a], Michael Cunningham[a], Nurtan A. Esmen[b], Thomas A. Hall[c], Margaret L. Phillips[c]

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Available online 22 August 2006

Abstract

We conducted an historical cohort study to investigate the mortality experience of industrial workers potentially exposed to chloroprene (CD) and other substances, including vinyl chloride (VC), with emphasis on cancer mortality, including respiratory system (RSC) and liver. In 1999, the International Agency for Research on Cancer (IARC) classified CD as a possible carcinogen (Group 2B); VC was classified in 1987 as a known human carcinogen (Group 1).

Subjects were 12,430 workers ever employed at one of two U.S. industrial sites (Louisville, KY (n=5507) and Pontchartrain, LA (n=1357)) or two European sites (Maydown, Northern Ireland (n=4849) and Grenoble, France (n=717)), with earliest CD production dates ranging from 1942 (L) to 1969 (P). Two sites (L and M) synthesized CD with the acetylene process that produced VC exposures. We determined vital status through 2000 for 95% of subjects and cause of death for 95% of the deaths. Historical exposures for individual workers were estimated quantitatively for CD and VC. Workers ever exposed to CD ranged from 92.3% (M) to 100% (G); to VC from 5.5% (M) to 22.7% (L). We computed standardized mortality ratios (SMRs) (using national and regional standard populations) in relation to selected demographic, work history and exposure factors. We used worker pay type (white or blue collar) as a rough surrogate for lifetime smoking history.

For the combined cohort, SMRs (95% CIs) for all causes combined, all cancers combined, RSC and liver cancer were, respectively, 0.72 (0.69–0.74), 0.73 (0.68–0.78), 0.75 (0.67–0.84) and 0.72 (0.43–1.13). Site-specific (L, M, P and G, respectively) SMRs were: for all cancers combined: 0.75 (0.69–0.80), 0.68 (0.56–0.80), 0.68 (0.47–0.95) and 0.59 (0.36–0.91); for RSC: 0.75 (0.66–0.85), 0.79 (0.58–1.05), 0.62 (0.32–1.09) and 0.85 (0.41–1.56); for liver cancer: 0.90 (0.53–1.44) (17 deaths), 0.24 (0.01–1.34) (1 death), 0.0 (0–2.39) (no deaths) and 0.56 (0.01–3.12) (1 death). Among all workers ever exposed to CD, SMRs were: for all cancers combined: 0.71 (0.66–0.76); for RSC: 0.75 (0.67–0.84); for liver cancer: 0.71 (0.42–1.14). We also observed no increased mortality risks among cohort subgroups defined by race, gender, worker pay type, worker service type (short/long term), time period, year of hire, age at hire, duration of employment, the time since first employment, and CD or VC exposure status (never/ever exposed).

In summary, our study has many strengths and is the most definitive study of the human carcinogenic potential of exposure to CD conducted to date. We conclude that persons exposed to chloroprene or vinyl chloride at the levels encountered in the four study sites did not have elevated risks of mortality from any of the causes of death examined, including all cancers combined and lung and

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0009-2797/$ – see front matter
doi:10.1016/j.cbi.2006.08.011

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Chemico-Biological Interactions
Chemico-Biological Interactions 166 (2007) 301–316
www.elsevier.com/locate/chembioint

Mortality patterns among industrial workers exposed to chloroprene and other substances II. Mortality in relation to exposure

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Available online 22 August 2006

Abstract

As part of an historical cohort study to investigate the mortality experience of industrial workers exposed to chloroprene (CD) and other substances, including vinyl chloride monomer (VC), we analyzed mortality from all cancers combined, respiratory system (RSC) and liver cancer in relation to CD and VC exposures. Subjects were 12,430 workers ever employed at one of two U.S. sites (Louisville, KY (n=5507) and Pontchartrain, LA (n=1357)) or two European sites (Maydown, Northern Ireland (n=4849) and Grenoble, France (n=717)).

Historical exposures for individual workers were estimated quantitatively for CD and VC. For sites L, M, P and G, respectively, average intensity of CD exposures (median value of exposed workers in ppm) were 5.23, 0.16, 0.028 and 0.149 and median cumulative exposures (ppmyears) were 18.35, 0.084, 0.133 and 1.01. For sites L and M, respectively, average intensity of VC exposures (median value of exposed workers in ppm) was 1.54 and 0.03 and median cumulative exposures (ppmyears) were 1.54 and 0.094.

We performed relative risk (RR) regression modeling to investigate the dependence of the internal cohort rates for all cancers combined, RSC and liver cancer on combinations of the categorical CD or VC exposure measures with adjustment for potential confounding factors. We categorized exposure measures into approximate quartiles based on the distribution of deaths from all cancers combined. We also considered 5- and 15-year lagged exposure measures and adjusted some RR models for worker pay type (white/blue collar) as a rough surrogate for lifetime smoking history. All modeling was site-specific to account for exposure heterogeneity. We also computed exposure category-specific standardized mortality ratios (SMRs) to assess absolute mortality rates.

With the exception of a one statistically significant association with duration of exposure to CD and all cancers combined in plant M, we observed no evidence of a positive association with all cancers, RSC or liver cancer and exposure to CD and/or VC using both the unlagged and lagged exposure measures: duration, average intensity or cumulative exposure to CD or VC; time since first CD or VC exposure; and duration of CD exposure or time since first CD exposure in presence or absence of VC exposure. We observed elevated and statistically significantly elevated RRs for some analysis subgroups, but these were due to inordinately low death rates in the baseline categories. With the possible exception of all cancer mortality in plant G, our additional adjustment of RRs for pay type revealed no evidence of positive confounding by smoking.

We conclude that exposures to CD or VC at the levels encountered in the four study sites do not elevate mortality risks from all cancers, RSC or liver cancer. This conclusion is corroborated by our analysis of general mortality patterns among the CD cohort

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0009-2797/$ – see front matter
doi:10.1016/j.cbi.2006.08.012

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